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Embryology of *Adenosma bilabiatum* (Roxb.) Merr. (= *A. capitatum* Benth.) (Scrophulariaceae)

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ABSTRACT: Anthers are tetrasporangiate. Anther wall development is of the dicotyledonous type. The glandular tapetum is single-layered and exhibits dual origin. Microspore tetrads are tetrahedral. Pollen grains at the time of anther dehiscence are two-celled and triaperturate. Ovules are unitegmic, tenuinucellar and anatropous. Development of female gametophyte conforms to the *Polygonum* type. Fertilization is porogamous. Endosperm is celluar with a single-celled chalazal haustorium, while the micropylar haustorium is initially four-celled, but becomes single-celled and quadrinucleate by the breakdown of the walls of the haustorial cells. Embryo development conforms to *Onagrad* type. Seed coat is thin with a prominent epidermal layer.

KEY WORDS: Embryology, *Adenosma bilabiatum*, Scrophulariaceae.

INTRODUCTION

The tribe Gratioleae of the subfamily Antirrhinoideae of Scrophulariaceae includes nearly 39 genera (Wettstein, 1897). Embryologically, members belonging to this tribe show great diversity with regard to the structure and organization of endosperm haustoria.

The genus *Adenosma* R. Br. belonging to Gratioleae includes nearly 15 species and is distributed in Indomalaya, China and Australia (Willis, 1973). A perusal of literature of the Gratioleae reveals that the genus *Adenosma* is embryologically unknown. The present study on *A. bilabiatum* (Roxb.) Merr. (=*A. capitatum* Benth) was, therefore, undertaken and deals with the study on the development and structure of gametophytes, endosperm, embryo and seed coat.

MATERIALS AND METHODS

Adenosma bilabiatum (Roxb.) Merr. is an erect herb, growing up to 2 ft in height with blue flowers in dense terminal heads. Material for the present study was collected from Kochuveli (near Tiruvananthapuram), Kerala State and fixed in F. A. A. (Formalin 5 mL; Acetic acid glacial 5 mL; 70% Ethyl alcohol 90 mL). The fixed material was then dehydrated and embedded in paraffin wax. Sections were cut at 8-11 μm on a Lipshaw

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rotary microtome and were stained by 0.5% Haematoxylin-0.001% orange G in clove oil combination (Johansen, 1940).

RESULTS

Microsporangium, microsporogenesis and male gametophyte

The anthers are tetrasporangiate. The hypodermal archesporium, organized at each corner of the young anther, comprises of a plate of 4-6 cells in cross section (Pl. 1, Figs. 1, 2). Following a periclinal division, the archesporial cells delimit the primary perietal and primary sporogenous layer of cells respectively (Pl. 1, Fig. 3). The former, by further periclinal divisions contribute to the wall layers comprising of the endothecium, one middle layer and the tapetum (Pl. 1, Fig. 5). While the endothecium and middle layer arise as sister layers, the tapetum differentiates directly from the inner parietal layer and is of the glandular type. It exhibits dual origin and as a consequence, a complete sheath of tapetum is established around the sporogenous cells (Pl. 1, Figs. 4, 5; Pl. 6, Fig. A). The tapetal cells become binucleate by about the time the pollen mother cells undergo meiosis (Pl. 1, Fig. 6, 7). However, by the time the uninucleate microspores are formed, the tapetum along with the middle layer become disorganized. At the time of releasing pollen, the endothecial cells become prominent as they undergo enlargement in size and develop fibrous bands of thickening, while the cells of the anther epidermis stretch tangentially and appear conspicuous (Pl. 1, Fig. 9). The pollen mother cells which differentiate from the sporogenous cells show normal meiosis, following simultaneous cytokinesis and produce tetrahedral microspores tetrads (Pl. 1, Figs. 10-12).

Pollen grains of adjacent microsporangia of an anther lobe are shed through a single opening formed as a consequence of breakdown of separating cell layers between them (Pl. 1, Fig. 8). The mature pollen grains are 2-celled and posses a triaperturate exine (Pl. 1, Figs. 13, 14).

Megasporangium, megasporogenesis and female gametophyte

The ovules are unitegmic, tenuinucellar, anatropous and are borne on the protruding placentae in each chamber of the bilocular ovary.

The female archesporium is single-celled and hypodermal in position (Pl. 2, Fig. 1). The archesporial cell after undergoing enlargement in size directly functions as the megaspore mother cell (Pl. 2, Fig. 2). Following meiosis in the mother cell, a dyad and a linear megaspore tetrad are formed respectively (Pl. 2, Figs. 3, 4). While the lower megaspore remains functional, the upper three megaspores exhibit early signs of degeneration. The nucleus of the functional megaspore undergoes three successive free nuclear divisions to organize an eight nucleate embryo sac comprising of the egg apparatus, three antipodal cells and a central cell containing two polar nuclei (Pl. 2, Figs. 4-9).

The mature embryo sac is straight with a broader micropylar part and a tubular chalazal portion. The micropylar part of the gametophyte slightly protrudes through the ovule (Pl. 2, Figs. 8, 9). Fusion of the polar nuclei takes place much before the gametophyte reaches fertilizable maturity. An endothelium surrounds the lower half of the female gametophyte at maturity (Pl. 2, Fig. 9).

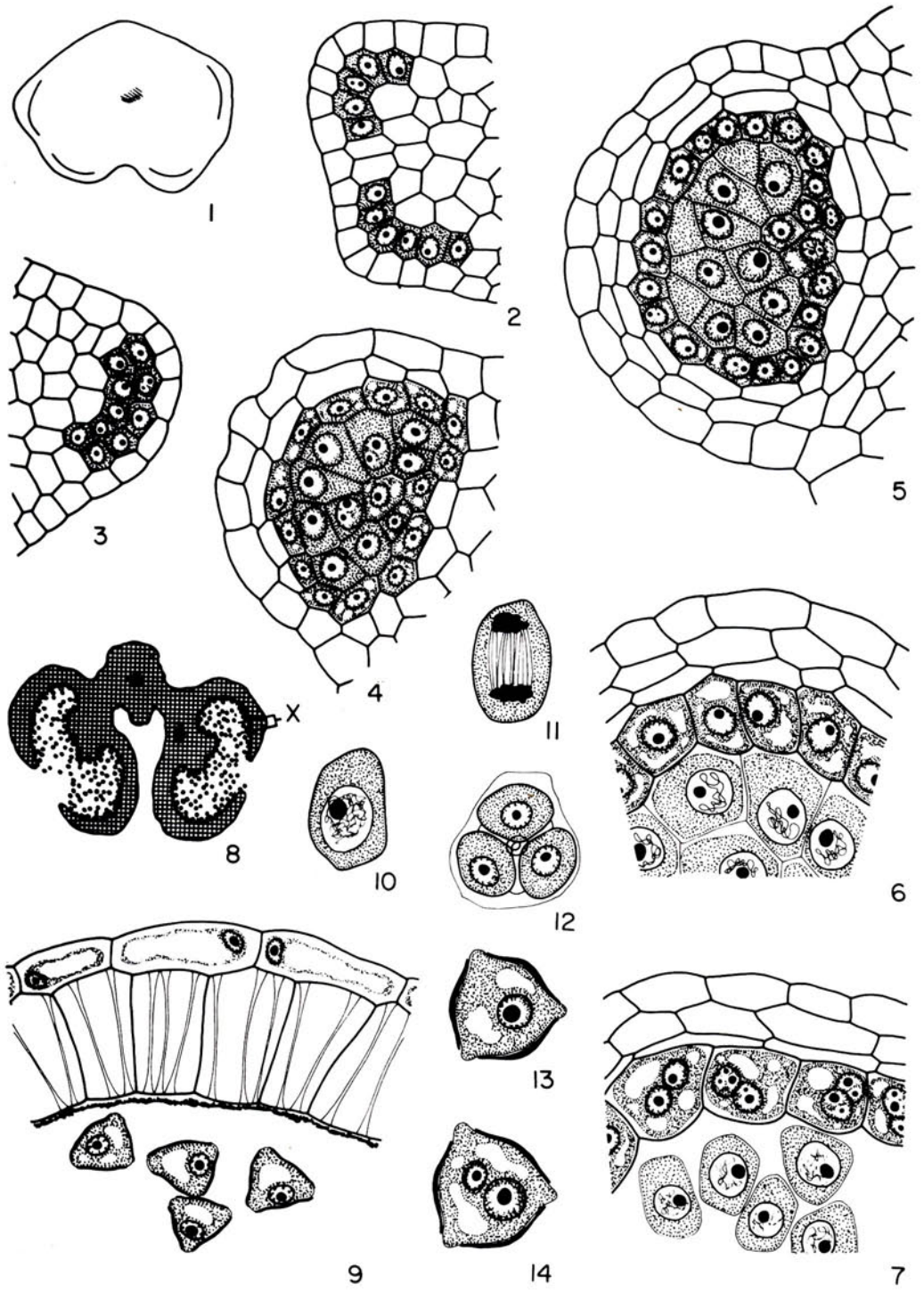

Plate-1. Microsporangium and male gemetophyte in *Adenosma bilabiatum.* Fig. 1. Outline of young anther in transection to show sites of microsporangia, x200. Fig. 2. A part of young anther in transection showing hypodermal archesporial plate of cells of adjacent microsporangia, x572. Figs. 3-5. Show stages in the development of microsporangium wall layers. Note dual origin of tapetum (fig. 3, x560, Figs. 4, 5, x635). Figs. 6, 7. A. Part of microsporangium wall in transection at pollen mother cell stage to show nuclear behaviour in tapetal cells, x735. Fig. 8. Outline of mature anther in transection x55. Fig. 9. Part marked X in Fig. 8 enlarged to show fibrous thickening in the endothecial cells x850. Fig. 10. A pollen mother cell x890. Fig. 11. Meiosis-I in the pollen mother cell x890. Fig. 12. A tetrahedral microspore tetrad x890. Fig. 13. Uninucleate pollen grain x890. Fig. 14. Mature bi - celled pollen grain x890.

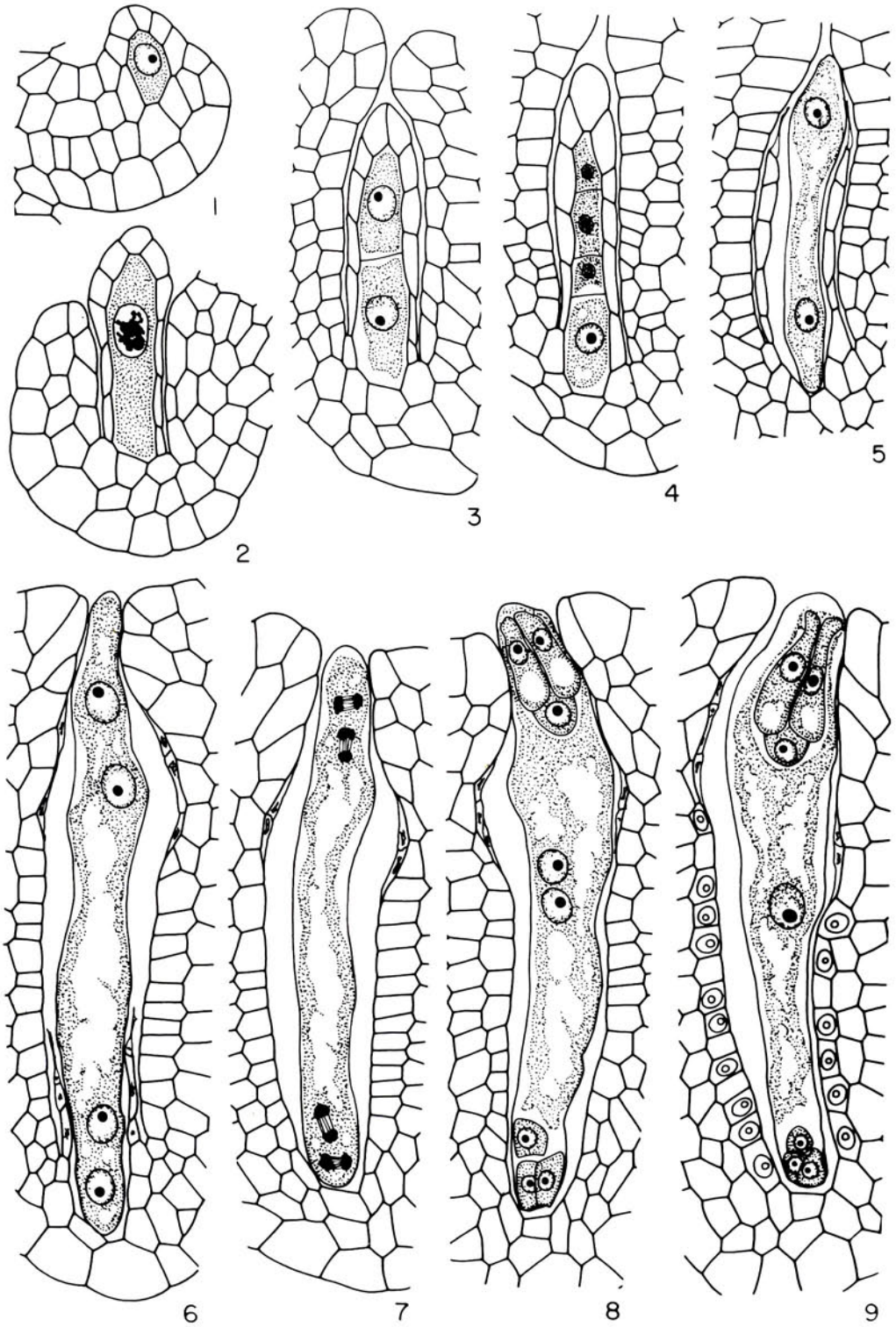

Plate-2 Female gametophyte development in *Adenosma bilabiatum*. Fig. 1. Ovular primordium to show hypodermal archesporial cell, x745. Fig. 2. Megaspore mother cell, x745. Fig. 3. Dyad, x745. Fig. 4. Linear megaspore tetrad. Note the functional chalazal megaspore, x745. Fig. 5. 2 nucleate embryo sac. Note the breakdown of nucellar envelope at micorpylar part, x745. Fig. 6. 4 nucleate embroyo sac. Note level of its micropylar end, x745. Fig. 7. Synchronous nuclear divisions in the 4 nucleate embryo sac, x745. Fig. 8. An organsied 8 nucleate embryo sac, x745. Fig. 9. Mature embyo sac. Note the endothelial coverage, x745.

Fertilisation

The path of entry of the pollen tube into the ovules is through the micropyle.

Endosperm

The first division of the endosperm mother cell is transverse resulting in two superposed primary endosperm chambers (Pl. 3, Figs. 2, 3). The lower primary chalazal endosperm chamber does not undergo any further divisions but directly organizes the chalazal haustorium (Pl. 3, Figs. 3-8). It extends downwards by breaking down cells along its path and invariably the terminal end of this haustorium comes to lie abutting the epidermal layer of the seedcoat. The chalazal haustorium remains single-celled and uninucleate throughout the period of its activity (Pl. 3, Fig. 8; Pl. 4, Fig. 12; Pl. 6, Fig. C). However, it degenerates much earlier to that of the micropylar haustorium.

The primary micropylar endosperm chamber, on the other hand, undergoes two vertical divisions at right angles to one another forming a tier of four circumjacent arranged cells (Pl. 3, Figs. 4, 5). A transverse division in these cells results in the delimitation of two superposed tiers of four cells each. While the upper tier of four cells organize the micropylar haustorium, the lower tier of cells contribute to the endosperm proper (Pl. 3, Fig. 6). The four uninucleate cells of the upper tier become conspicuous by their dense cytoplasm and prominent nucleus and organize the micropylar haustorium (Pl. 3, Figs. 7-9). The haustorium at its peak of activity shows breakdown of the separating walls of its cells rendering the haustorium single-celled and quadrinucleate (Pl. 4, Figs. 10, 11; Pl. 6, Fig. B). Meanwhile, the initial cells of the endosperm proper by further divisions produce a massive homogeneous endosperm tissue (Pl. 4, Figs. 7-9).

Embryo

The first division of the zygote occurs only after the initial establishment of the endosperm and is transverse resulting in a two-celled proembryo comprising of the terminal cell (ca) and a basal cell (cb) (Pl. 5, Figs. 1, 2). Following a vertical division in ca and a transverse division in cb, a T-shaped proembryo is formed. While the cell cb by further transverse divisions produce cells d, f and ci, the cell ca after undergoing two vertical division at right angles to one another organize the quadrant tier q (Pl. 5, Fig. 3). Meanwhile, the cell ci by transverse division gives rise to cells d, f, n and n'. Cells f, n & n' organize the embryonal suspensors (Pl. 5, Figs. 4-9). The cells of the quadrant tier after a transverse division organise the octants wherein the cells are disposed in two superposed tiers of four cells each-l and l' (Pl. 5, Fig. 4).

By further divisions in the cells of the tier l and l', the embryo assumes a globular and heart shape, eventually to produce a mature dicotyledonous embryo (Pl-5, Figs. 5-16). The histogens become differentiated during the early globular stage of embryogenesis.During further development, cells derived from tier l contribute to the shoot tip/epicotyl (pvt) and cotyledonary primordia (pco) while those of l' contribute to the hypocotyledonary part (phy) of the embryo. The root hypophysis, is chiefly contributed by the derivatives of cell d and comprises of the cells of the root cortex (iec) and root cap (co) (Pl. 5, Figs. 5-9).The mature embryo has well developed cotyledons (cot), a prominent shoot tip (pvt), a hypo-cotyledonary part (phy) and the root hypophysis (iec and ico) (Pl. 5, Fig. 13).

Seed coat

The seed coat is constituted by the cells of the single integument which initially comprise of 4-5 cell layers (Pl., 4 Fig. 1). During early stages of seed development the cells undergo enlargement in size (Pl. 4, Figs. 7-9). As the seed attains maturity the epidermal cells of the seed coat become conspicuous by their large size, while the underlying cells including the endothelial cells undergo considerable stretching, as a result of which they become compressed or crushed. Meanwhile, the epidermal cells acquire band-like thickenings on their radial walls and warty depositions on their inner tangential walls (Pl. 4, Figs.10, 12). The mature seed coat is thin and comprises mainly of the prominent epidermal layer with the inner cell layers including the endothelium appearing crushed (Pl. 4, Figs. 13, 14).

DISCUSSION

The anther, in *Adenosma bilabiatum* is tetrasporangiate and the development of microsporangium wall conforms to the Dicotyledonous type (Davis, 1966). This is true in all the other investigated taxa of Gratioleae. The glandular tapetum is single-layered and exhibits dual origin, a feature that has been recorded for many other taxa of Scrophulariaceae and thus in general agreement with the view expressed by Periasamy and Swamy (1966). However, in *Mimulus ringens* (Arekal, 1965) and *Mimulus guttatus* (Urs and Jayaraj, 1997) the tapetum exhibits bilayered condition at certain places due to periclinal division occuring in its cells. The tapetal cells in *A. bilabiatum* do not exhibit dimorphic behaviour as observed in *Mimulus* (Arekal, 1965; Urs and Jayaraj, 1997).

The development of female gametophyte in all the taxa of Gratioleae thus far investigated, including the present study, is fairly consistent and conforms to the Polygonum type (Maheshwari, 1950). The mature gametophyte of *Adenosma bilabiatum* slightly protrudes out through the micropyle. This is in contrast to the condition met with in certain taxa such as *Vandellia* (Krishna Iyegar, 1940; Yamazaki, 1954) and *Torenia* (Balicka Iwanoska, 1899; Krishna Iyengar, 1941; Guilford and Fisk, 1952; and Yamazaki, 1954), where the micropylar part of the female gametophyte is extra micropylar. The antipodal cells are small in size and thin-walled. The endothelium surrounds the lower narrow tapering end of the female gametophyte only.

The endosperm in all the Gratioleae so far examined including the present study is ab initio cellular type with well developed haustoria. The chalazal haustorium is single-celled and uninucleate. Such a feature has also been recorded for *Limosella aquatica* (Svensson, 1928), *Limnophila heterophylla* (Krishna Iyengar, 1939), *Torenia* (Guilford and Fisk, 1952; Yamazaki, 1954), *Bacopa hamiltoniana* (Safeulla and Govindu, 1950). *Bonnaya spp.* (Yamazaki, 1954), *Lindenbergia indica* (Pal, 1958), *Artanema sesamoides* (Urs, 1989) and *Limnophila sessilis* (Urs and Arekal, 1995-96), occasionally, however, in *Torenia spp.* (Krishna Iyengar, 1941, Guilford and Fisk, 1952) and *Artanema sesamoides* (Urs, 1989) the haustorium is single-celled and binucleate. Such a condition is not met with in the present study.

The micropylar haustorium in *Adenosma bilabiatum* comprises of four uninucleate cells which are circumjacent. A similar feature has also been consistently noticed in the other investigated members of the tribe such as *Limosella aquatica* (Svensson, 1928) *Limnophila*

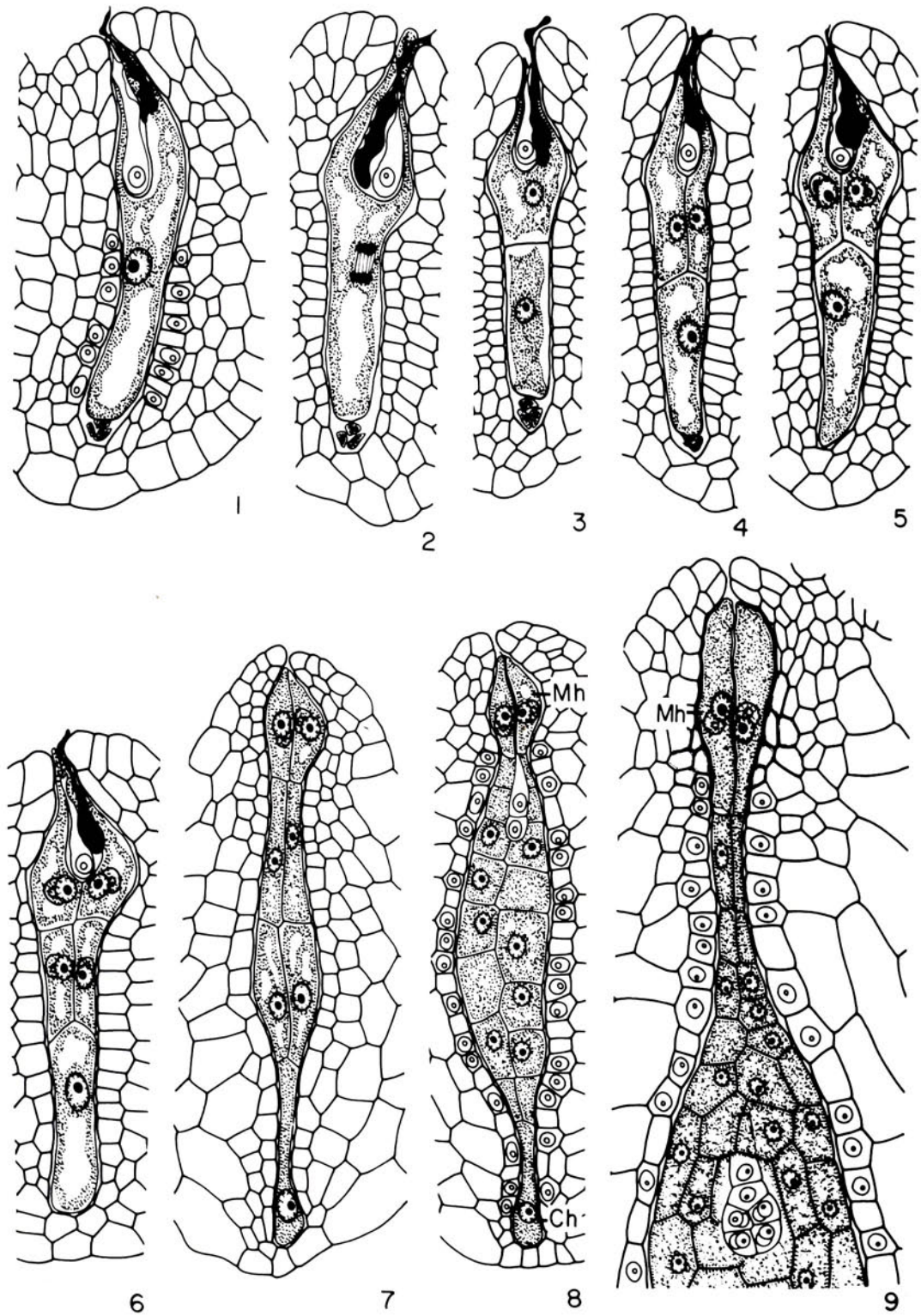

Plate-3. Endosperm development in *Adenosma bilabiatum*. Fig. 1. Fertilized embryo sac. Note degenerating antipodal cells and persistent remains of pollen tube. Fig. 2-8. Show stages in the development of endosperm and organization of endosperm haustoria (all figs. x465). Fig. 9 A part of seed in transection at octant stage of embryo showing 4-celled micropylar haustorium x465. (Ch-Chalazal haustorium; Emb-Embryo; End-Endosperm; Int-Integument; Mh-Micorpylar haustorium).

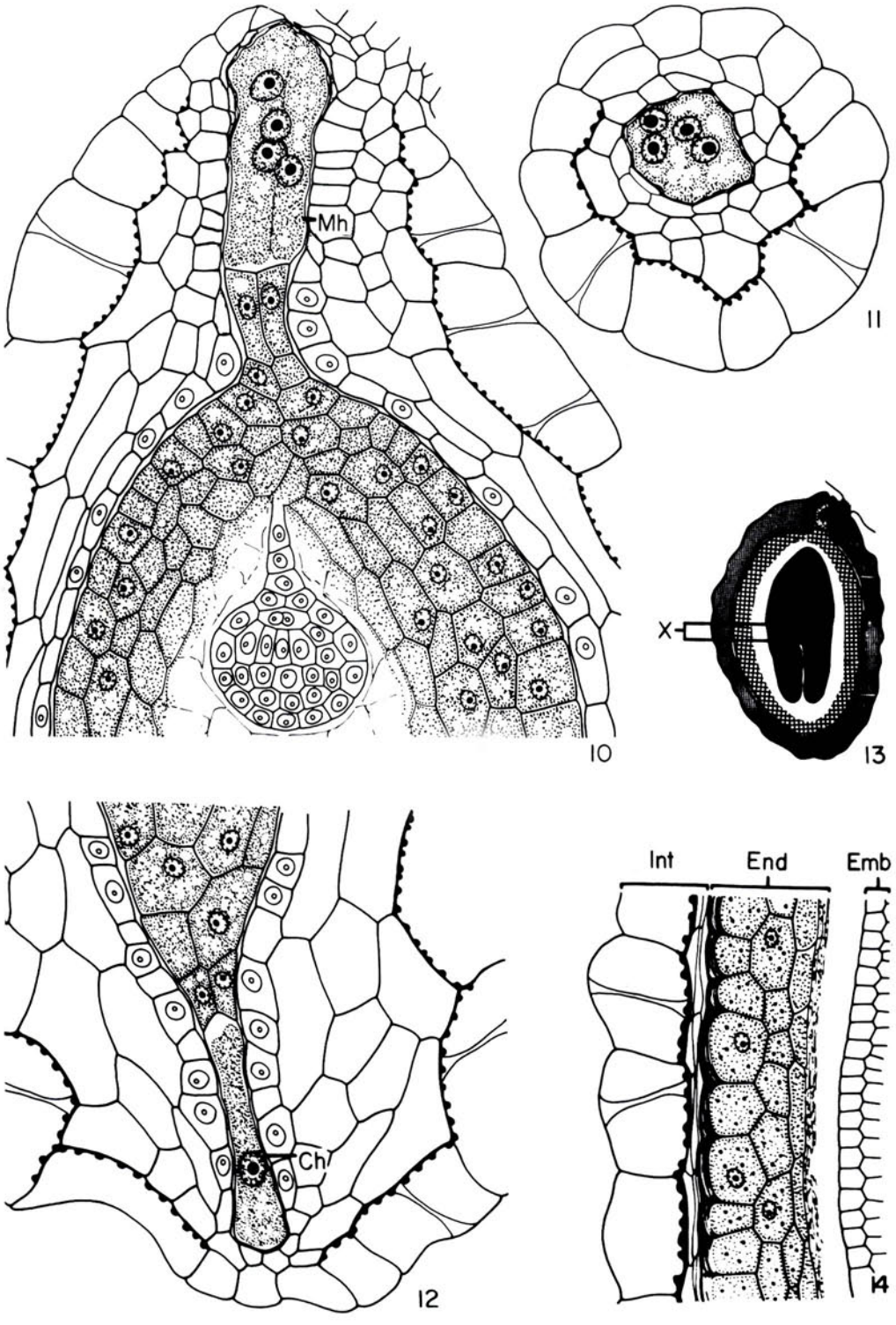

Plate-4. Endosperm in *Adenosma bilabiatum* (Contd.) Figs. 10 & 12. A part of seed at globular stage of embryo to show extent of micropylar and chalazal endosperm haustoria, x455. Fig. 11. Transection of seed to show fusion micropylar haustorial cells, x455. Fig. 13. Outline of ripe seed in longitudinal section, x65. Fig. 14. Part marked X in Fig. 13 enlarged to show structure of seed coat. Note uneven thickenings and radial bands on walls of epidermal cells, x270. (Ch-Chalazal haustorium; Emb-Embryo; End-Endosperm; Int-Integument; Mh-Micorpylar haustorium).

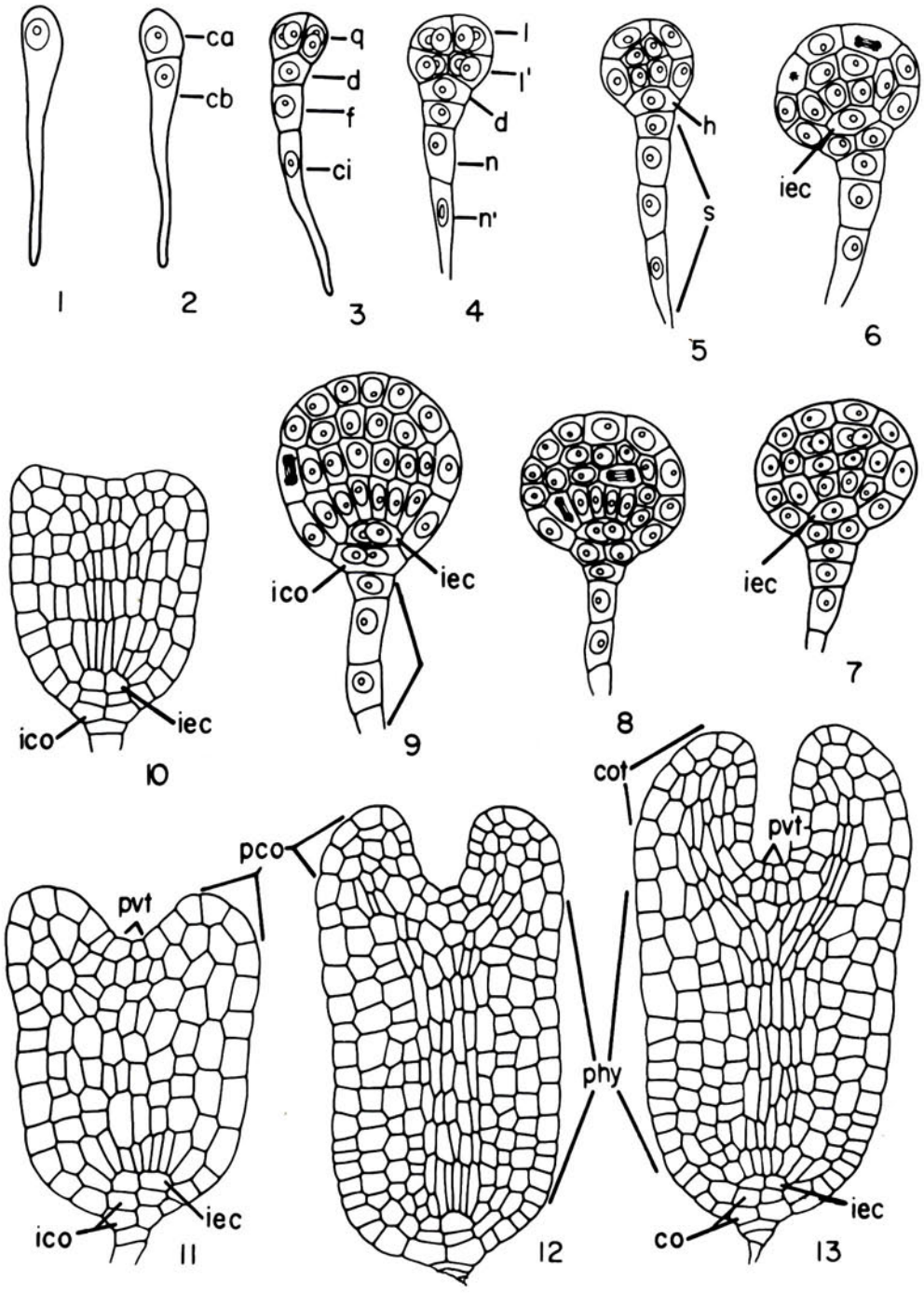

Plate-5. Embryo development in *Adenosma bilabiatum*. Fig. 1. Zygote, x580. Fig. 2. 2-celled proembryo, x580. Fig. 3. Quadrant embryo, x580. Fig. 4. Octant embryo, x580. Figs. 5-9. Show stages in the development of globular embryo, x580. Fig. 10-11. Show heart-shaped embryo, x580. Figs. 12, 13. Mature embryo, x350. ca = apical cell; cb = basal cell; d = hypophyseal cell; f, ci, n, n' = daughter cells of cb; iec = initial cells of root cortex; ico, co = initial cells of root cap; l = terminal tier of cells of octant; l' = subterminal tier of cells of octant; pco, cot = cotyledonary region; phy = hypocotyledonary region; pvt = epicotyl/shoot tip; q = quadrants; s = suspensor.

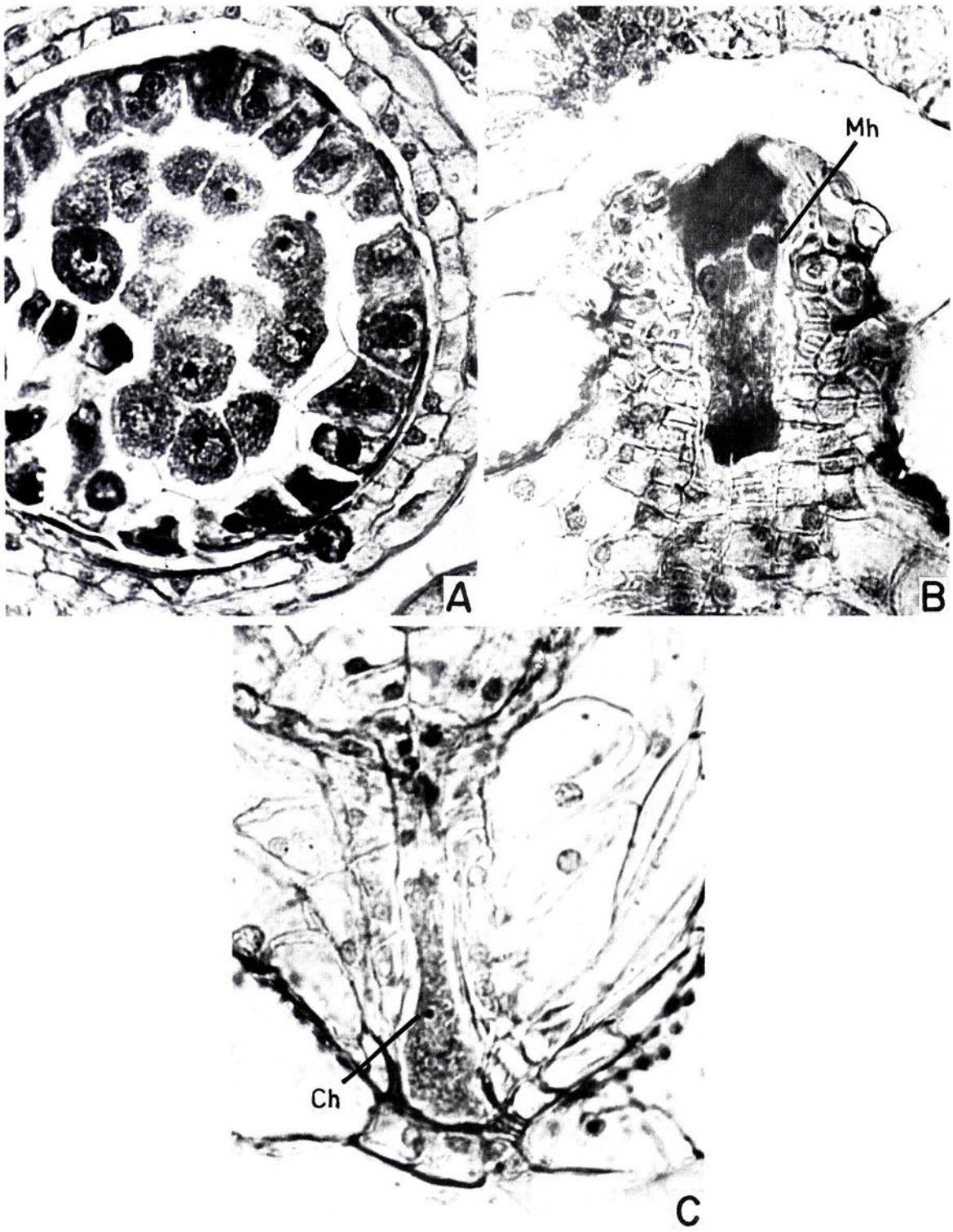

Plate-6. Photo micrographs. Fig. A. Transection of Microsporangium at pollen mother cells stage to show binucleate tapetal cells, x860. Fig. B. A part of longitudinal section of seed to show micropylar haustorium (Mh), x680. Fig. C. A part of of chalazal region of seed in longitudinal section showing the haustorium (Ch), x860.

heterophylla, Stemodia viscosa (Krishna Iyengar, 1939), *Illysanthes spp.* (Krishna Iyengar, 1940; Raghavan and Srinivasan, 1941; Yamazaki, 1954), *Lindernia pyxidaria, Bonnaya verbenaefolia, B. ruelloides, Vandellia setulosa* (Yamazaki, 1954), Lindernia hyssopioides (Arekal *et al.*, 1970), Artanema sesamoides (Urs, 1989) and *Limnophila sessilis* (Urs and Arekal, 1995-96). Further, as in *Limnophila heterophylla, Stemodia viscosa, Vandellia hirsuta, V. scabra, Torenia cordifolia, T. hirsuta* (Krishna Iyengar, 1939, 1940, 1941) *Torenia fournieri* (Guilford and Fisk, 1952) and *Limnophila sessilis* (Urs and Arekal, 1995-96), in the present study also, the separating walls in between the haustorial cells break down rendering the haustorium single-celled and quadrinucleate. On the contrary, in *Dienostema violacea* (Yamazaki, 1953) the haustorial cells exbit partial fusion.

The endosperm surface, as in a majority of Gratioleae so far studied is smooth and non-ruminate, although, in certain species *Vandellia, Torenia*, (Krishna Iyengar, 1940, 1941; Guildford and Fisk, 1952; Yamazaki, 1954), and *Artenema* (Urs, 1989) the endosperm is uneven and ruminate. As in all the other Gratioleae the seed coat in *Adenosoma bilabiatum* consist of a prominent epidermal layer and degenerating layers of integumentary cells including the endothelium.

Embryo development in all the members of Gratioleae including the present study is similar and corresponds to the *Onagrad* type (Johansen, 1950). Therefore, embryologically *A. capitatum* conforms to the rest of the gratioleae investigated so far.

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Adenosma bilabiatum (Roxb.) Merr. (玄參科) 的胚胎學

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摘　　要

花藥為四孢子型。花藥壁之發育為雙子葉型。分泌型營養層為單層細胞且為雙重起源。四分小孢子為四分錐形體。花藥開裂時花粉粒為二細胞期且具三個萌芽孔。胚珠為單層珠被，薄珠心，倒生胚珠型。雌配子體的發育為蓼型。受精作用是經由珠孔。胚乳是細胞型具單細胞合點端吸器，而珠孔端吸器起源時為四細胞，但由於吸器細胞之細胞壁的瓦解而成為具四核之單細胞。胚胎發育為柳葉菜型。種皮薄具一明顯表皮層。

關鍵詞：胚胎學，*Adenosma bilabiatum* (Roxb.) Merr.，玄參科。

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