Embryology of *Micrargeria wightii* Benth. (Scrophulariaceae)

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MS received 1 October 1986; revised 30 June 1987

Abstract. The anther is tetrasporangiate. The young microsporangium wall comprises of an epidermis, a fibrous endothecium, a middle layer and a layer of glandular tapetum of binucleate cells. The tapetum is of dual origin and exhibits morphological dimorphism. The development of the female gametophyte conforms to the Polygonum type. An endothelium surrounds the middle part of the embryo sac at maturity. The endosperm is *ab initio* cellular. The chalazal haustorium is single-celled and binucleate. The micropylar haustorium is highly aggressive. To begin with it has two binucleate cells, which subsequently fuse forming a single-celled, quadrinucleate bulbous body. Branched hypha-like tubular processes extending down along the conducting strand of the developing seed reaching almost the chalaza arise from it. The development of the embryo conforms to Crucifer type. The mature seed coat consists of 2–3 layers of cells together with the degenerating remains of the endothelium. The systematic position of the tribe Gerardeae in the subfamily Rhinanthoideae has been evaluated on embryological grounds.

Keywords. Embryology; Scrophulariaceae; Gerardeae; *Micrargeria*; systematics.

1. Introduction

Embryological studies have been of considerable use in the assessment of relationships among taxa of the Scrophulariaceae. The contributions of Arekal (1963a, b, 1964, 1966), Arekal *et al* (1970) and Hakki (1977) in this regard are quite significant. Nevertheless, the investigations on the tribe Gerardeae of the subfamily Rhinanthoideae are meagre (Michell 1915; Krishna Iyengar 1937, 1940, 1942; Srinivasan 1946; Tiagi 1956; Arekal 1964; Vijayaraghavan and Ratnaparkhi 1972, 1973; Arekal and Raju 1967, 1976–77; Nagendran *et al* 1980) being confined to only 7 taxa despite 26 genera (Wettstein 1897) and 150 species comprising the tribe. Among the subfamily Rhinanthoideae, the Gerardeae stands in between the Digitaleae and Rhinanthaeae. While Digitaleae includes only autotrophs, the Rhinanthaeae contains exclusively parasitic taxa. The present study was carried out to understand the ontogeny and organisation of the seed in the tribe Gerardeae and to ascertain whether this tribe finds an intermediate position in between Digitaleae and Rhinanthaeae, on embryological grounds. The genus *Micrargeria* which is represented in the Indian sub-continent by a single taxon *M. wightii* Benth., is one of the uninvestigated taxa belonging to this tribe and is a root hemi-parasite mostly found on grasses in open areas of scrub jungles.

2. Materials and methods

The present study is based on buds, flowers and fruits at various stages of development collected at the foot of Chamundi Hills, Mysore city and also near Yelwal, 7 km from Mysore city. Customary methods were followed during dehydration and

embedding. Sections were cut between 8–15 μm and stained in Heidenhain's Iron alum haematoxylin with Erythrosin in clove oil as counterstain.

3. Observations

3.1 *Microsporangium and male gametophyte*

A cross section of a very young anther reveals 4 strips of hypodermal archesporial cells (figure 1). The cells are conspicuous by their comparatively large size, dense cytoplasm and prominent nuclei. A periclinal division of the cells results in the organisation of the primary parietal and primary sporogenous layers (figure 2). The former, through a similar division gives rise to two layers of cells (figure 3). Of these the one bordering the primary sporogenous cells directly differentiates into the tapetum, while the other produces a middle layer and the endothecium (figure 4). Meanwhile, cells adjoining the sporogenous cells on the connective side of the anther become conspicuous by acquiring dense vacuolate cytoplasm and function as the tapetum. The sporogenous tissue, therefore, becomes ensheathed by a continuous layer of glandular tapetum (figure 4).

During further development of the sporangium, the tapetal cells on the connective side elongate and enlarge markedly in contrast to the rest of its cells (figure 5). Nevertheless, all the cells become binucleate after a free nuclear division, especially when the microspore mother cells are organised within the microsporangium. The tapetum gets depleted of its contents and disappears along with the middle layer after the formation of pollen grains. At maturity of the anther, the endothelial cells enlarge in size and acquire band-like thickenings (figures 6–7).

The primary sporogenous cells do not undergo many divisions and generally a single row of cells is noted in transections of the microsporangium (figure 5). The spore mother cells undergo meiosis to result in tetrahedral tetrads of microspores (figures 8–10). Cytokinesis of the spore mother cells is simultaneous.

The microspores of the tetrad enlarge in size, become spherical and separate apart. Vacuolisation of their cytoplasm occurs (figure 11). The centrally located nucleus moves to a side and divides resulting in a small densely protoplasmic generative cell and a larger vacuolate vegetative cell. By this time, the adjacent microsporangial cavities of the anther coalesce and the pollen grains of the two sporangia are released through a common opening (figure 6). At shedding, the grains are 2-celled and triaperturate with a thick exine (figure 12).

3.2 *Megasporangium and female gametophyte*

A large number of finger-like ovular primordia arise on the massive axile placental humps of a young bilocular ovary. They give rise to tenuinucellate, unitegmic and hemianatropous to nearly anatropous ovules.

The young ovular primordium organises a conspicuous large hypodermal archesporial cell much before the integument appears. After enlargement and elongation, the archesporium directly functions as the megaspore mother cell (figures 13–14). After meiotic division, it gives rise to a linear tetrad of megaspores (figure 15).

Figures 1-12. Microsporangium and male gametophyte in *M. wightii*. 1. Outline t. s. of a very young anther showing the sites of microsporangia ($\times 89$). 2. T. s. of young microsporangium showing primary sporogenous and primary parietal layers ($\times 540$). 3-5. T. s. of developing microsporangia ($\times 540$). Note single row of microspore mother cells surrounded by a layer of binucleate tapetal cells in figure 5. 6. Outline t. s. of mature anther ($\times 240$). 7. Part marked X in figure 6 enlarged to show wall layers ($\times 810$). 8-10. Development of tetrahedral microspore tetrad from a mother cell ($\times 810$). 11. Uninucleate microspore ($\times 810$). 12. Mature 2-celled pollen grain ($\times 810$).

The chalazal megaspore of the linear tetrad enlarges and functions while the rest degenerate (figure 16).

The functional megaspore elongates as its cytoplasm becomes vacuolate and its centrally located nucleus undergoes a free nuclear division forming two daughter nuclei (figure 17). The 2-nucleate embryo sac thus established, soon organises a 4-nucleate embryo sac by a simultaneous division of its nuclei (figures 18–20). After one more nuclear division, the embryo sac becomes 8-nucleate with a quartet of nuclei at each pole. The micropylar quartet contributes to the egg apparatus and the micropylar polar; the chalazal quartet takes part in the formation of 3 antipodal cells and the chalazal polar. The two polar nuclei meet near the egg apparatus (figure 21) and ultimately fuse together forming the secondary nucleus.

A fully organised embryo sac (figure 22) has a broader micropylar part and its tapering terminal part lodges the egg apparatus. The two synergids are posteriorly vacuolate while the egg has an anterior vacuole. The secondary nucleus is located near the broader part of the micropylar region. The 3 antipodal cells are prominent and they gradually breakdown during early seed development.

The single-layered nucellus becomes gradually crushed and absorbed during development. Consequently, the embryo sac comes to lie in direct contact with the inner epidermis of the integument, which organises an integumentary tapetum at the lower part of the female gametophyte only, save the broader micropylar region and the extreme chalazal tip (figure 22).

3.3 *Endosperm*

Fertilisation is porogamos. The first division of the endosperm mother cell is transverse and it occurs much earlier than that of the zygote, initiating two superposed primary endosperm chambers (figures 23–24). The primary chalazal chamber soon becomes binucleate after a free nuclear division and directly functions as the chalazal haustorium (figures 25–26). By a transverse division, the primary micropylar chamber organises two tiers of two cells each. The upper tier of cells contributes to the micropylar haustorium while the lower one includes initials of the endosperm proper (figure 27).

A free nuclear division in the micropylar haustorial cells renders them binucleate. Both these cells soon fuse together and extend out laterally as a quadrinucleate bulbous body towards the conducting strand of the developing seed by destroying the cells on its path (figure 28). The subsequent extension of the haustorium is intercellular. Its further extension is by the production of narrow branched tubular processes which descend down along the conducting strand of the seed (figure 29). The 4 nuclei of the haustorium generally enter into the enlarged lateral extension, increase in size and become hypertrophied. The micropylar haustorial activity is much more aggressive than that of the chalazal haustorium (figures 29–30) and it ceases only when the seed ripens.

Meanwhile, the two initial cells of the endosperm proper divide a few times transversely and give rise to a biseriate row of cells. By further anticlinal and transverse divisions, a homogenous mass of endosperm tissue is produced (figures 29–30). When the seed ripens, most of the central core of the endosperm tissue gets consumed by the developing embryo. The remaining endosperm cells accumulate

Figures 13-22. Female gametophyte development in *M. wightii* ($\times 590$). 13. Young ovular primordium with archesporial cell. 14. Megaspore mother cell. 15. Synchronous nuclear division in the superposed dyad cells. 16. Linear tetrad of megaspores. Note the upper 3 megaspores in the process of degeneration. 17. Nuclear division in the functional megaspore. Note breakdown of cells of the nucellar envelope. 18. 2-Nucleate embryo sac. 19. Nuclear division in the 2-nucleate sac. 20. 4-Nucleate embryo sac. 21. Organised 8-nucleate embryo sac. 22. Mature embryo sac.

Figures 23–28. Endosperm development in *M. wightii* ($\times 560$). **23.** Fertilised embryo sac. Note degenerated synergid and remains of pollen tube. **24.** 2-celled endosperm. **25.** 3-celled endosperm with dividing nucleus in the chalazal chamber. **26.** 3-celled endosperm with binucleate chalazal chamber. **27.** 5-celled endosperm. **28.** Initial organisation of endosperm. Note lateral extension of fused micropylar haustorial cells. (Note persistent antipodal cells in figures 23–25).

reserve food materials in the form of densely staining granules. The outer tangential wall of the outermost layer of the endosperm acquire heavy lamellated thickenings. By this stage, the cells of the seed coat become vertically stretched. The large prominent outer epidermal cells of the seed coat build a sheet of peg-like thickenings on the inner face of the outer tangential wall (figures 31–32).

Figures 29–32. Endosperm in *M. wightii*. 29–30. L. s. of micropylar and chalazal parts of the same seed at young globular stage of embryo show aggressive haustorial activity of endosperm ($\times 380$). 31. Outline l. s. of ripe seed ($\times 27$). 32. Part marked X in figure 31 enlarged to show details of seed coat ($\times 380$). (Ch, Chalazal haustorium; Emb, embryo; End, endosperm; Int, integument; Mh, micropylar haustorium).

3.4 Embryo

The zygote elongates and its division is followed by a transverse wall forming two unequal cells (figures 33–35). The smaller terminal cell *ca* then engenders two juxtaposed cells after a vertical division, while the lower basal cell *cb* gives rise to two superposed cells *m* and *ci* by a transverse division (figure 36). Thus the resulting tetrad is T-shaped and conforms to the A_2 category of Souèges (1948) (figure 36).

The vertical wall at right angles to the previous one is soon laid in the juxtaposed cells of *ca* to form the quadrant *q* (figure 37). The quadrant soon organises the octant after a transverse division, the cells being disposed in two tiers, *l* and *l'* (figure 38) of 4 each. Simultaneously, the cell *ci* forms *n*, *n'*, while *m* also divides transversely forming *d* and *f* respectively.

During subsequent development, the cells of the octant undergo a periclinal division delimiting an outer layer of protoderm initials from a central group of cells (figure 39). The initials of the protoderm undergo further anticlinal divisions. Meanwhile, the central group of cells of tier *l'* after vertical divisions delimit the provascular zone (figures 40–41). After repeated anticlinal and periclinal divisions, the derivatives of the tier *l* organise the stem tip *pvt*, and the cotyledonary primordia *pco*, while those of *l'* establish the hypocotyledonary part *phy* of the embryo (figures 42–44). The cotyledonary primordia form two elongated cotyledons by further divisions of cells.

Concomitant with the cell divisions in the upper tier of the octant, the cell *d* which is the uppermost cell of the suspensor abutting the tier *l'* or its derivative functions as the hypophyseal cell, *h* (figure 39). After a transverse division, the hypophyseal cell cuts off a small lenticular cell towards the inner side which functions as the initial cell of the root cortex *iec*, and an outer, the initial cell of the root cap *ico* (figures 40–41). By two vertical divisions one at right angles to the other, the initial cells of the root cortex and root cap organise a plate of 4 cells each. As the embryo attains maturity, the plate of 4 cells constituting the initials of the root cap cells divide transversely engendering two plates of 4 cells in each tier, while the initials of the root cortex do not divide (figures 43–45).

The mature embryo has two well developed cotyledons, a stem tip (a well organised hypocotyledonary part) and a radicular part (figure 45).

4. Discussion

The structure and development of the microsporangium in *M. wightii* is essentially similar to that of *Striga orobanchoides* and *S. euphrasioides* (Tiagi 1956), *Gerardia pedicularia* (Arekal 1964), *Alectra thomsoni* (Vijayaraghavan and Ratnaparkhi 1973) and *Buchnera hispida* (Arekal and Raju 1976–77). Although a majority of Gerardeae so far studied has a tetrasporangiate anther, in *B. hispida* (Arekal and Raju 1976–77) it is only bisporangiate. The bisporangiate state is considered as derived through a simple process of elimination of one half of the anther (Tieghem 1903).

The endothecium and the middle layer of the microsporangium wall in the present study are sister layers. However, Tiagi (1956) pointed out that the middle layer and the tapetum are sister layers in *Striga*. Further, Vijayaraghavan and Ratnaparkhi (1973), reported that in *A. thomsoni*, the wall of the microsporangium comprises of an

Figures 33-45. Embryo development in *M. wightii*. 33. Zygote ($\times 550$). 34-36. Establishment of the proembryonal tetrad ($\times 550$). 37. Quadrant ($\times 550$). 38. Octant embryo ($\times 550$). 39-42. Stages in the development of globular embryo ($\times 550$). 43-44. Heart-shaped stage of developing embryo ($\times 550$). 45. Mature embryo ($\times 330$).

epidermis, the endothecium, two middle layers and the tapetum; the outer middle layer and the endothecium being sisters while the inner middle layer and the tapetum in turn also being sisters. Apparently the mode of organisation of the microsporangium wall in Gerardeae is variable.

Micrargeria is more like *Buchnera* (Arekal and Raju 1976-77) having a single-layered tapetum while *Striga* (Tiagi 1956) is similar to *Gerardia* (Arekal 1964) in possessing a two-layered tapetum, although only occasionally. Nonetheless, in all the taxa of the tribe so far studied including the present one, the tapetum is of dual origin. Similarly, the radial elongation of tapetal cells on the connective side and their marked conspicuousness during meiosis of pollen mother cells are common in members of this tribe. The 2-celled pollen grains at shedding as noted in *Micrargeria* have also been recorded for *Striga* (Tiagi 1956), *Gerardia* (Arekal 1964), *Alectra* (Vijayaraghavan and Ratnaparkhi 1973) and *Buchnera* (Arekal and Raju 1976-77).

The ontogeny and organisation of the female gametophyte in the present investigation conforms to the *Polygonum* type as in other investigated members of the tribe. However, Vijayaraghavan and Ratnaparkhi (1972) have noted the co-existence of both *Polygonum* and *Allium* types of embryo sac in *A. thomsoni*.

The organisation of an endothelium around the narrow part of the embryo sac as noted in the present study has also been reported in *Sopubia* (Krishna Iyengar 1937, 1940), *Striga* (Tiagi 1956; Nagendran *et al* 1980), *Gerardia* (Arekal 1964) and *Alectra* (Vijayaraghavan and Ratnaparkhi 1972). However, in *B. hispida* (Arekal and Raju 1976-77) it surrounds only the middle part of the embryo sac.

The endosperm in *M. wightii* is *ab initio* cellular. Its early ontogeny is closely similar to that of *Rhamphicarpa longiflora* (Krishna Iyengar 1942), *G. pedicularia* (Arekal 1964) and *B. hispida* (Arekal and Raju 1976-77) although in *Gerardia* (Arekal 1964) the nuclear division in the micropylar chamber is followed by an incomplete vertical wall.

The chalazal haustorium noted in the present study is single-celled and binucleate as in *R. longiflora*, *Centranthera hispida* (Krishna Iyengar 1942), *S. orobanchoides* and *S. euphrasioides* (Tiagi 1956), *G. pedicularia* (Arekal 1964), *A. thomsoni* (Vijayaraghavan and Ratnaparkhi 1972), *B. hispida* (Arekal and Raju 1976-77) and *Striga densiflora* (Nagendran *et al* 1980). The extension of the haustorium as a tubular process towards the conducting strand of the seed by breaking down cells has also been observed in the other investigated taxa of the tribe. However, in *C. hispida* (Krishna Iyengar 1942) this haustorium remains inactive and assumes a bulbous shape.

The micropylar haustorium in *Micrargeria* consists of two binucleate cells which fuse together at an early stage and extend out laterally towards the conducting strand as a single-celled, quadrinucleate bulbous body. Further, extension of this haustorium is intercellular by the production of narrow branched tubular processes which descend down along the conducting strand of the seed. Such a feature has also been recorded in *Sopubia* (Krishna Iyengar 1937, 1940; Arekal and Raju 1967). However, in *R. longiflora* (Krishna Iyengar 1942) and *G. pedicularia* (Arekal 1964) the tetranucleate haustorium extends towards the chalaza along the conducting strand of the developing seed as a single large tubular process. On the other hand, in *C. hispida* (Krishna Iyengar 1942), *A. thomsoni* (Vijayaraghavan and Ratnaparkhi 1972) and *B. hispida* (Arekal and Raju 1976-77) the two binucleate haustorial cells neither fuse nor significantly extend out into the surrounding integumentary tissue during

seed development. Furthermore, in *C. hispida* (Krishna Iyengar 1942) and *A. thomsoni* (Vijayaraghavan and Ratnaparkhi 1972) secondary haustoria arise from the cells of the endosperm located below the micropylar haustorium, a feature quite unique in itself.

It is obvious, therefore, that in Gerardeae the ontogeny and organisation of the micropylar haustorium varies significantly, ranging from a state of inactivity to a highly active tubular extensions reaching almost the chalazal end of the developing seed.

The development of the embryo in *Micrargeria* conforms to that of *Capsella bursa-pastoris* (Souèges 1948) as in the other investigated taxa of the tribe.

The Gerardeae share several features in common with the tribe Rhinanthae particularly in the endosperm ontogeny and organisation of the endosperm haustoria. The primary micropylar endosperm chamber divides by a vertical wall (often incomplete) before the origin of the micropylar haustorium and the initials of the endosperm proper, a character also noticed in the Rhinanthae (Arekal 1963). Further, the marked extension of the micropylar haustorium and its aggressive activity as noted in *Rhamphicarpa* (Krishna Iyengar 1942), *Gerardia* (Arekal 1964) and *Micrargeria* of the present study have also been noticed in *Pedicularis* (Berg 1954), *Euphrasia*, *Melampyrum* and *Orthocarpus* (Arekal 1963) and *Rhinanthus* (Tiagi 1967). Nonetheless, the presence of a nonaggressive micropylar haustorium which does not extend out as seen in *Buchnera* (Arekal and Raju 1976-77) is a feature similar to the state noted in most of the Digitaleae, although in the latter the sequence of wall formation during initial development of endosperm is significantly different. Therefore, the placement of the tribe Gerardeae in between Digitaleae and Rhinanthae by Wettstein (1897) appears to be justified on the basis of embryology.

The parasitic habit noted in some members of Gerardeae is a general character of members of Rhinanthae. Moreover, some of the Gerardeae such as *Buchnera*, *Sopubia* and *Micrargeria* are facultative parasites indicating a transition from autotrophy to heterotrophy.

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