Contribution to the embryology of *Melampyrum pratense* L.

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Abstract. In *Melampyrum pratense* the anther is tetrasporangiate and anther wall consists of epidermis, fibrous endothecium, a transitory middle layer and glandular tapetum. The division of pollen mother cell is simultaneous. At shedding, the pollen grains are 2-celled, 3-colpate and spheroidal.

The gynoecium is superior and the ovary is bicarpellary, syncarpous with two tenuinucellate, unitegmic ovules in each locule.

A coenomegaspore with 1+3 arrangement of nuclei develops into a 7-nucleate embryo sac. The female gametophyte is tetrasporic and 7-nucleate.

The *ab initio* cellular endosperm is haustorial. The chalazal haustorium is large, binucleate and highly aggressive. The 8-16 nucleate micropylar haustorium is unicellular with 2-4 tubular processes, one of which is longer and aggressive.

Keywords. *Melampyrum pratense*; embryology

1. Introduction

The tribe Rhinantheae of the subfamily Rhinanthoideae, Scrophulariaceae is regarded as a heterogeneous assemblage of genera (Lawrence 1951). Arekal (1963) and Tiagi (1965, 1966) have reviewed embryological literature pertaining to this tribe. According to Schmid (1906) the development of female gametophyte in *Melampyrum silvaticum* and *M. pratense* is of the Polygonum type. Arekal (1963) opines that in *M. lineare* the embryo sac is tetrasporic and 7-nucleate. Greilhuber (1973) made an extensive karyomorphological study on embryo sac and endosperm of six European species of *Melampyrum* and concluded that the embryo sac development corresponds to tetrasporic, biphasic, bipolar and belongs to the modified Drusa type I. The present investigation dealing with embryology of *Melampyrum pratense* was undertaken because of differences of opinion on the development of female gametophyte and the information on the endosperm is wanting in the literature. The observations presented are based on the material collected by Professor Krupko of Poznon, Poland and kindly passed on to us.

2. Observations

2.1 Microsporangium and male gametophyte

A transverse section of an young anther reveals the 4-lobed structure (figure 1).
2.2 Ovary and ovules

The gynoecium is superior, syncarpous and the ovary is bilocular with two ovules in each locule (figures 17-19), on an axile placenta. The tongue-like nectary is located on one side at the base of the ovary. The ovular primordia are initiated as outgrowths from the axile placenta. While the upper pair of ovular primordia gives rise to semicampylotropous ovules during subsequent development, the lower pair ends as hemianatropous ovules. The ovules, therefore, exhibit heterotropy, the upper pair having the micropyyle directed towards the septum and the lower facing the ovary wall. Both the types of ovules are tenuinucellate with a massive integument and comparatively long funiculus (figures 20-24).

2.3 Megasporangium and female gametophyte

Usually a single densely-cytoplasmic, large-nucleated, hypodermal archesporial cell organises very early in the ovular primordium (figure 25). The archesporial cell enlarges and functions directly as megaspore mother cell (figure 26). Occasionally two such cells have been observed (figure 27). Meiosis I of the megaspore mother cell is not followed by a cell wall, consequently dyad cells are not formed. The two resulting daughter nuclei divide simultaneously and produce 4-megaspore nuclei, the micropylar one of which is larger (figure 28). Vacuolization of the cytoplasm sets in by the time the coenomegaspore exhibits 1+3 arrangement of nuclei (figure 29). The micropylar nucleus, divides while those at the chalazal part do not (figure 30). The embryo sac now exhibits a 2+3 arrangement of nuclei (figure 31). By
Figures 1-16. Microsporangium and male gametophyte in *Melampyrum pratense*. 1. Outline t.s. of an young anther, $\times 475$. 2. T.s. of microsporangial primordium with hypodermal archesporial cells, $\times 1500$. 3 and 4. Parts of the anther lobes showing primary parietal and primary sporogenous layers, $\times 1500$. 5. Part of microsporangium; note uniseriate glandular tapetum (te), a middle layer (ml), a layer of endothecium (en) sporogenous tissue (spt), $\times 1050$. 6 Outline of t.s. of mature anther, $\times 105$. 7. Part of t.s. of mature anther lobe showing endothelial thickening, $\times 1050$. 8-12. Meiotic divisions of pollen mother cell, $\times 3000$. 13. Tetrahedral tetrad of microspores, $\times 3000$. 14-15. Uninucleate microspore, $\times 3000$. 16. 2-celled mature pollen grain, $\times 3000$. 
another free nuclear division at the micropylar end the embryo sac ends into the 7-nucleate stage with 4+3 arrangement. Three of the four nuclei in the micropylar part of the embryo sac enter into the formation of egg apparatus of two synergids and an egg, the fourth functioning as polar nucleus (figures 32-33). One of the chalazal megaspore nuclei functions as a chalazal polar nucleus. It is smaller and stains very poorly compared to the micropylar polar nucleus and moves upwards and meets its counterpart but does not fuse with it (figures 33-34). The other megaspore nuclei at the chalazal end may either remain as such or may contribute to the organisation of antipodal cells.

During the development of embryo sac the surrounding nucellar cells get crushed and two to four degenerated cells persist at the micropylar end of the embryo sac. As the embryo sac attains maturity the inner epidermal cells of the integument surrounding the micropylar part of the embryo sac become obliterated and absorbed. This coincides with the hypodermal cells becoming densely cytoplasmic and functioning as the integumentary tapetum. The embryo sac during its development extends towards the chalaza breaking down the surrounding cells.

The mature embryo sac (figure 35) is more or less cylindrical with a bulbous chalazal region. It develops 2-4 narrow tubular processes at the micropylar end. The egg apparatus consists of two synergids and a pear-shaped egg. The two polar nuclei do not fuse and a typical secondary nucleus does not therefore result. The chalazal megaspore nuclei contribute to the formation of antipodals.

2.4 *Endosperm*

The endosperm is *ab initio* cellular. The first division of the endosperm mother cell (figure 36) occurs much earlier than the division of the zygote and is followed by a curved wall (figure 37). The resulting chambers are unequal. The larger chalazal chamber incorporates the polar nucleus which does not fuse with the counterpart either before or during double fertilization. The large nucleus in the chalazal chamber undergoes a free nuclear division producing two daughter nuclei (figure 38). By this time the incorporated nucleus in the chalazal chamber degenerates. Meanwhile the division of the nucleus of the micropylar chamber is followed by an incomplete vertical wall (figure 38). The binucleate chalazal cell directly functions as the chalazal haustorium. The two nuclei in the micropylar chamber divide simultaneously to form two juxtaposed cells above the chalazal haustorium, and a large binucleate cell towards the micropylar end (figure 39). The former serves as initials of endosperm proper and the latter functions as the micropylar haustorium.

The young chalazal haustorium (figures 39-41) remains two-nucleate, enlarges enormously consuming the surrounding cells. It is highly aggressive and retains its activity for a long time. In a well developed seed the hypertrophied nuclei often break up into fragments.

The young binucleate micropylar haustorium with its tubular extensions become 8-16 nucleate by free nuclear divisions (figures 40-42). One of the tubular processes on the raphe side become larger in size, elongates and extends towards the funiculus without damaging any integumentary cells of the seed. The irregular-shaped nuclei enter into this tube. The activity of the haustorium continues for a long period.
Figures 25-35. Megasporogenesis and female gametophyte in *Melampyrum pratense*. 25. L.s. of an ovular primordium with a hypodermal archesporial cell, × 1050. 26: A megaspore mother cell, × 1050. 27. Double megaspore mother cells, × 1050. 28-29. Coenomegaspores with 1+3 nuclei arrangement, × 1050. 30. 2+3 nucleate embryo sac, × 1050. 31-34. Organized embryo sac at different stages; note the antipodal cells in figure 34, × 1050. 35. A mature embryo sac, × 1500.
Figures 36-42. Endosperm in *Melampyrum pratense*. 36. Fertilized embryo sac, × 1500. 37. 2-celled endosperm; note the presence of chalazal polar nucleus, X 1500. 38. 3-celled endosperm, × 1500. 39. 5-celled endosperm, × 1500. 40-41. Endosperm with binucleate chalazal haustorium and micropylar haustorium, × 1500. 42. Upper part of old seed showing 16-nucleate micropylar haustorium and endosperm tissue; × 1050.
The two initials of the endosperm proper undergo a series of transverse and longitudinal divisions producing a massive endosperm tissue.

3. Discussion

The mode of initiation and general organisation of microsporangium in the present study is similar to the other investigated Rhinantheae. The anther wall, in addition to the epidermis, comprises a glandular tapetum, a transitory middle layer and a fibrous endothecium. As in other species of *Melampyrum* the mature pollen grains are 2-celled, 3-colpate and spheroidal.

The development of the female gametophyte in *Melampyrum pratense* is tetrasporic and 7-nucleate as in *Melampyrum lineare* (Arekal, 1963). Schmid (1906) who studied *M. pratense* and *M. silvaticum* reported a polygonum type of female gametophyte in the two taxa. The present study has revealed beyond doubt that a coenomegaspore is consistently formed before it develops into an embryo sac. The persistent degenerated nucellar cells above the developing embryo sac probably have misled Schmid (1906) to interpret the development as monosporic. Greilhuber (1973) who made karyomorphological studies of six European species of *Melampyrum* found tetrasporic female gametophyte in every one of them and confirmed the work of Arekal (1963). The organization of mature embryo sac in *Melampyrum pratense* is similar to that of *M. lineare* (Arekal, 1963). In the former the number of tubular processes developed at the micropylar end of the sac is 2-4 as against 6-8 in *M. lineare* (Arekal, 1963).

Unlike the rest of the angiosperms the integumentary tapetum in the genus *Melampyrum* is epidermal in origin at its base and hypodermal in the rest of the region. This is borne out by ontogenetic events noted during ovule development. Arekal (1963) who investigated *M. lineare* had believed that it was entirely hypodermal in origin. Balicka-Iwanowska (1899) and Schmid (1906) stated that the endothelium belonged to the inner epidermis of the integument. Tiagi (1965) who recently investigated *M. arvense*, *M. nemerosum*, *Rhinanthus major* and *R. serotinus* (Tiagi, 1965, 1966) did not make an ontogenetic study of the developing endothelium, he opined that the endothelium belongs entirely to the inner epidermis of the integument. The present study has revealed that the lowermost part of the endothelium belongs to the epidermis and the rest of the part belongs to the layer below the epidermis, since the tubular extensions of the embryo sac arise and penetrate deeper into the surrounding integumentary tissue.

The endosperm is cellular from inception. The first division of the endosperm mother cell in all the investigated members of Rhinantheae is transverse and the second is vertical in the primary micropylar chamber. The present study, therefore, corresponds to the development and general organization of endosperm to *M. lineare* (Arekal, 1963), *M. arvense*, *M. nemerosum*, *Rhinanthus major* and *R. serotinus* (Tiagi, 1965, 1966).

The highly aggressive, large, binucleate, chalazal haustorium of *M. Pratense* basically corresponds to that of *M. lineare* (Arekal, 1963), *M. arvense*, *M. nemerosum*, *Rhinanthus major* and *R. serotinus* (Tiagi, 1965, 1966). The hypertrophied nuclei often break up into fragments. As in other members of Rhinantheae the bulbous haustorium extends towards the conducting strand bringing about the degeneration
of surrounding cells and its activity is observed for a considerable period during post-fertilization stages.

The organization of the micropylar haustorium and its later behaviour conforms to that of *M. lineare* (Arekal, 1963). The occurrence of micropylar haustorium has been recorded in the investigated species of *Melampyrum* (Balicka-Iwanowska, 1899; Schmid 1906; Arekal, 1963 and Tiagi 1965). Unlike *M. lineare*, *M. pratense* resembles *M. silvaticum* (Schmid, 1906). The haustorium appears to draw nutrients directly from the living integumentary cells. The number of haustorial nuclei in *M. pratense* is 8-16 while in others it is commonly four. As in other species of *Melampyrum* the hypertrophied nuclei enter into the larger of the tubes, irrespective of numbers.

References


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