A contribution to the embryology of *Trachelospermum fragrans* Hook. f. (Apocynaceae)

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Abstract. Embryology of *Trachelospermum fragrans* Hook. f. is described. The anther wall consists of the epidermis, fibrous endothecium, 2-3 middle layers and parietal tapetum. Rarely, endothecium shows 2 layers. The tapetum is uniseriate and remains uninucleate throughout. Simultaneous cytokinesis in pollen mother cells produces tetrahedral and isobilateral tetrads. Degeneration of few pollen grains is recorded. Pollen grain is triporate and 3-celled with few starch grains at anthesis. The ovule is hemianatropous, rarely anatropous, unitegmic and tenuinucellate. The chalaza megaspore of the linear tetrad develops into the 8-nucleate embryo sac of the Polygonum type. Synergids are hooked and the ephemeral antipodals appear egg-like. Fertilization is porogamous. Triple fusion occurs a little earlier than syngamy. The endosperm *ab initio* is free nuclear and later becomes cellular. Endosperm haustorium is present. Embryogeny conforms to the Solanaceae type.

Keywords. *Trachelospermum fragrans;* embryology; Apocynaceae.

1. Introduction

The embryological work on Apocynaceae was reviewed by Schnarf (1931), and noteworthy contributions to the embryological studies of the family were made by Fyre and Blodgett (1905), Guignard (1917a, b), Andersson (1931), Meyer (1938) and Rau (1940). While summarising the embryological data on Apocynaceae, Davis (1966) mentioned the controversy concerning the nature of division of pollen mother cells. Similarly, due to the occurrence of different types of embryogenesis, Maheshwari Devi (1971) called Apocynaceae 'a heterogeneous group' and pointed out the need for further investigations on this aspect. Subsequently, only a few contributions to the embryological literature of the family have been made by Murty and Chauhan (1966), Maheshwari Devi (1970, 1971, 1974), Bhasin (1971) and Lamba (1974, 1976). In view of the scant existing information on the embryology of the family Apocynaceae, the present findings on *Trachelospermum fragrans* Hook. f. are of interest.

2. Material and methods

The study material was collected from Simla hills and fixed in formalin–acetic alcohol. Tertiary-butyl alcohol series was used for dehydration before embedding the material in paraffin wax (m.p. 56–58°C). Serial sections (8–14 μ), were stained in Delafield haematoxylin as well as safranin-fast green combination of which the first gave the best results.
3. Observations

3.1 Microsporogenesis and male gametophyte

Anther is bithecous. In young anther, the cells of the hypodermal archesporium undergo periclinal divisions to produce an outer layer of primary parietal cells and an inner layer of sporogenous cells (figures 1, 2). Further periclinal divisions in the parietal layer result in the formation of a fibrous endothecial layer, 2-3 middle layers and a single layered tapetum (figures 3, 4). The tapetum is of secretory type. The tapetal cells remain uninucleate throughout and possess 1 or 2 prominent vacuoles (figure 4). The anther wall is thus constituted by an epidermis, a fibrous endothecium, 2-3 middle layers and a tapetum (figure 4). Rarely, the fibrous endothecium appears 2-layered (figure 5). As wall layers mature, epidermal cells appear more prominent. However, the endothecium becomes inconspicuous in the old anther. Disorganisation of the middle layers and the tapetum starts at the tetrad stage of the microspores. A few microspores also appear disintegrating soon after cytokinesis (figure 6).

Divisions of the sporogenous tissue result in the formation of pollen mother cells which undergo meiotic divisions (figure 7) followed by simultaneous cytokinesis to produce tetrahedral (figure 8) and isobilateral (figure 9) tetrads. Young pollen grains are globular or somewhat spherical in outline with vacuolated cytoplasm. Before division the nucleus is seen shifting toward periphery. Division of the nucleus is followed by a lenticular wall to form the generative and vegetative cells (figure 10). Subsequently, the lenticular wall disappears. Mature pollen grains are triporate and 3-celled during shedding (figure 11). The exine is quite smooth. Only a few starch grains are recorded in a fully-developed pollen grain.

3.2 Megasporogenesis and female gametophyte

The ovary is superior and bicarpellary with 3–many ovules in each carpel. The ovule is hemianatropous, rarely anatropous, unitegmic and tenuinucellate. The rapid elongation of the integument makes the 8-nucleate embryo sac deep-seated. The hypodermal archesporial cell (figure 12) enlarges considerably to function directly as the megaspore mother cell (figure 13). It undergoes meiotic divisions and produces a linear tetrad of megaspores (figure 14). Rarely, T-shaped tetrads (figure 15) are also observed. The upper three megaspores degenerate while the chalazal one becomes functional (figures 14, 15).

With the enlargement of the chalazal megaspore its nucleus undergoes three successive mitotic divisions to produce 2, 4 and 8-nucleate embryo sacs (figures 16–18). It is at the two-nucleate stage that there occurs the disorganization of the epidermal layer of the nucellus surrounding the embryo sac. A distinct integumentary tapetum has not been observed. Mature embryo sac (figure 18) is broader in the middle, and narrow and slightly bent at the chalazal end. The uninucleate synergids are pear-shaped, hooked and with a prominent basal vacuole. Antipodals are elongated and somewhat egg-like, uninucleate and ephemeral but their remnants persist till the formation of the secondary nucleus. Rarely, 8-nucleate embryo sac is seen showing 3-micropylar and 5-chalazal free nuclei (figure 19). Mature embryo sac contains a considerable amount of starch grains which are consumed after fertilization.
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3.3 Pollination and fertilization

The pollen tube enters the embryo sac through the micropyle. One male gamete lies near the egg and the other near the polar nuclei (figure 20). Later, the polar nuclei come to lie adjacent to the egg apparatus. The male nucleus fuses with polar nuclei just before the other male nucleus fuses with the egg.

3.4 Endosperm

The fertilized embryo sac grows considerably in size. The primary endosperm nucleus moves to the centre where it undergoes free nuclear divisions. Due to the formation of a central vacuole, the nuclei are shifted towards the periphery of the embryo sac (figure 21). Cell formation in the endosperm begins from periphery at the 4-6-celled proembryo stage. By the time a heart-shaped embryo is formed, the entire embryo sac becomes cellular (figure 22). A few endosperm cells appear 2-nucleate (figure 23). Endosperm gets consumed by the developing embryo. The seeds are ex-albuminous. Endosperm haustoria (figure 22) are seen developing at the chalazal end of the embryo sac.

3.5 Embryo

A transverse division of the enlarged fertilized egg results in the formation of a terminal cell, ca and a basal cell, cb (figure 24). The second transverse division in both the cells results in a linear proembryo of 4-cells, l, l', m and ci (figures 25, 26). Another transverse division in each of the two cells l and l' produces 4 cells termed l1, l2, l1' and l2' (figures 27, 28). Due to the two vertical divisions, which are at right angles to one another, in the cells l1, l2 and l1', quadrants are formed (figures 29, 30) which undergo divisions in all directions to form the embryo proper (figures 31, 32). The tier l1 eventually gives rise to two leafy cotyledons and the tier l2 to the hypocotyledonary region. The derivatives of the tier l1 contribute to the root proper. Subsequent transverse divisions in one or more cells of the l2, m and ci result in the formation of a single-layered suspensor of 4–7 cells (figure 32). The development of the embryo is thus of the Solanad type. Mature embryo shows well-defined root cap, hypocotyl, root-axis, plumule and two cotyledons (figure 33).

4. Discussion

The development of the anther wall in *Trachelospermum fragrans* conforms to the Dicotyledonous type (Davis 1966). The present study shows 1–2 layered fibrous endothecium and 2–3 middle layers. This is contrary to Maheshwari Devi (1971) who reported a multiple endothecium and 10–14 middle layers in *Volacanga foetida* Rolfe. The endothecium becomes inconspicuous in the old anther. A similar condition was reported in *Cerbera odollam* Gaertn and *Vallaris heyneii* Spreng by Rau (1940). An uniseriate parietal tapetum has been found in this plant as in *Thevetia nervosula* Juss and *Alstonia scholaris* R. Br (Meyer 1938), *Cerbera odollam* (Rau 1940), *Locherna pusilla* K. Schum (Murty and Chauhan 1966), *Catharanthus roseus* (Linn.) G. Don, *C. puzillus* (Murr.) G. Don, *Holarrhena antidysenterica* Wall., *Rauolfia serpentina* L. Benth. ex Kurz, *Volacanga foetida* and *Carissa spinarum* Linn. (Maheshwari Devi 1971, 1974). The
Figures 1-33. Stages in the development of male and female gametophytes, endosperm and embryo in *T. fraoraas* Hook. f.
1. archesporial cells; 2. primary parietal and primary sporogenous layers; 3. wall layers and sporogenous layer; 4. epidermis, endotheecium, middle layers, tapetum and pollen mother cells; 5. a portion of the 2-layered fibrous endotheecium; 6. young microspores, the disorganising microspores and tapetum; 7. microspore mother cells in meiotic division; 8, 9. tetrahedral and isobilateral tetrads of microspores; 10. 2-celled young pollen grain; 11. 3-celled mature pollen grain; 12. LS ovule showing archesporial cell; 13. LS part of nucellus showing megaspore mother cell; 14, 15. linear and T-shaped tetrads of megaspores, note the functional chalazal megaspore; 16, 17. 2 and 4-nucleate embryo sacs, respectively; 18. organised 8-nucleate embryo sac; 19. abnormal embryo sac showing 3-micropylar and 5-chalazal nuclei; 20. upper portion of embryo sac showing triple fusion and syngamy; 21. LS of embryo sac showing nuclear endosperm; 22. LS of embryo sac showing cellular endosperm, heart-shaped embryo and endosperm haustorium; 23. a portion of endosperm showing 2-nucleate cells; 24-32. stages in embryogeny; 33. LS of mature embryo.

Abbreviations. t, tapetum; ft, fibrous thickenings; dm, disintegrating microspore; dt, disorganising tapetum; elo, egg-like antipodals; pt, pollen tube; emb, embryo; eh, endosperm haustorium; rc, root cap.
present study shows simultaneous cytokinesis of pollen mother cells. This observation corroborates with earlier findings of Andersson (1931), Meyer (1938), Rau (1940) and Maheshwari Devi (1971, 1974). As usual for Apocynaceae, pollen grains are shed at the 3-celled stage. However, anthesis at the 2-celled stage has been reported in *Catharanthus pusillus* by Bhasin (1971) and *Rauwolfia serpentina* by Lamba (1976). A few microspores have been seen disorganising soon after cytokinesis, presumably due to the failure of the tapetum to provide full nourishment to all the developing microspores in a sporangium.

As in all the other investigated members of Apocynaceae, the archesporial cell functions directly as the megaspore mother cell and forms a linear tetrad. A rare occurrence of $T$-shaped tetrads in this plant resembles with the earlier finding of Maheshwari Devi (1970, 1971) in *Catharanthus roseus*. As usual for the family, the chalazal megaspore develops into 8-nucleate embryo sac of the Polygonum type. A well-defined integumentary tapetum has been reported in *Amsonia tabernaemontana* Walt and *Rhazya orientalis* Decne by Andersson (1931), but in the present case such distinct tapetum has not been observed which confirms the earlier observations of Rau (1940) and Lamba (1974) on some other Apocynaceae. Abnormal embryo sac with 3 micropylar and 5 chalazal free nuclei, egg-like antipodals and hooked synergids constitute the important features of *T. fragrans*. Maheshwari Devi (1971) has also reported hooked synergids in *R. serpentina*. Disorganisation of epidermal layer of the nucellus surrounding the embryo sac starts at the 2-celled stage of embryo sac as in other investigated taxa of Apocynaceae (Rau 1940; Lamba 1974).

In *C. roseus* triple fusion occurs simultaneously with fertilization (Maheshwari Devi 1971). The present investigation shows that triple fusion takes place a little earlier than syngamy as in *Vallaris heyneii* (Rau 1940) and *Lochnera pusilla* (Murty and Chauhan 1966).

Endosperm development is of the nuclear type as reported by Murty and Chauhan (1966), and Maheshwari Devi (1971, 1974) in some other Apocynaceae. The presence of endosperm haustoria in Apocynaceae was first recorded in *Lochnera pusilla* by Murty and Chauhan (1966), later by Maheshwari Devi (1971, 1974) in *C. roseus*, *C. pusillus* and *Carissa spinarum* and is now being reported in *T. fragrans*. The development of embryo in this plant conforms to the Solanad type. *Trachelospermum fragrans* thus, closely resembles the hitherto investigated members of Apocynaceae.

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