MICROSPOROGENESIS AND THE DEVELOPMENT OF MALE GAMETOPHYTE IN TWO SPECIES OF *DIOSPYROS* L.

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Abstract: Development of the microsporangium, and the male gametophyte were studied in *Diospyros chloroxylon* Roxb. and *D. virgiana* L. The anther wall development is of the Dicotyledonous type and at maturity it consists of the epidermis, fibrous endothecium, 2-3 wall layers and a secretory tapetum which is uniseriate with uni- or binucleate cells. Connective tapetum is also differentiated. Pollen mother cells divide simultaneously and produce tetrahedral tetrads. Cytokinesis is by furrowing. Pollen grains are smooth, tricolporate and two celled at the time of liberation. Rarely 3-nucleate pollen grains are seen. Pollen degenerations are common in *D. virginiana.*

INTRODUCTION

A perusal of the previous literature on the family Ebenaceae has revealed that the embryological investigations in the family are very limited and incomplete. In fact there is very little information on the microsporogenesis and the development of the male gametophyte. *Diospyros* L. is a large genus with 500 species (Willis, 1966) and is mostly found in the warmer regions of the world. Embryological studies in this large genus are very meagre. Hence in the present study it is planned to investigate the developmental morphology of the microsporangium in two species of *Diospyros*, namely *D. chloroxylon* Roxb. and *D. virginiana* Linn.

MATERIAL AND METHODS

Flower buds of *D. chloroxylon* were collected from the Andhra University campus, Waltair and *D. virginiana* from Sim's Park, Ooty, Tamil Nadu, India. The material was fixed in FAA and customary methods of dehydration, infiltration and embedding were followed. The sections were cut between 4-10 μm in thickness and were stained in Delafield's hematoxylin.

OBSERVATIONS

Floral Morphology

D. chloroxylon is a small sized wild tree whereas *D. virginiana* is medium sized tree which is grown in gardens for its beautifil foliage. As the plants are dioecious male and female flowers occur on separate plants. Male flowers appear in axillary fascicles. They are small in size, white in colour, shortly pedicellate, tetramerous, regular and cyclic. Stamens are epipetalous, inserted and are about

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16 in their numbers (Fig. 1A). Each stamen has a short slender filament at the base and a dithecous, tetrasporangiate anther at the top.

Microsporangium, microsporogenesis and the male gametophyte

A very young anther consists of a homogenous mass of meristematic cells, which are enclosed by a single celled thick epidermis. It soon becomes four lobed (Fig. 1A, B). The archesporium is hypodermal in origin and consists of a plate of 3-8 cells (Fig. 1C, D). These cells appear in the four corners of an young anther when it assumes four lobed nature and they can be recognised by their larger nuclei and dense cytoplasm. The archesporial cells divide periclinally resulting in to an outer primary parietal layer and an inner primay sporogenous layer (Fig. 1E, F). The parietal layer undergoes one more periclinal division so that two layers are found below the epidermis, of which the inner one develops directly into a tapetum. The outar layer by further periclinal divisions produce three layers. The outer most layer of these three develop into an endothecium. The cells of the endothecium are large, tangentially flattened and when they mature they become radially elongated. By the time the sporogenous cells complete the meiotic divisions, fibrous bands of secondary wall material start appearing from the inner tangential walls. These thickenings attain their maximum development when the pollen grains are about to shed (Fig. 1J). The next two layers of cells just beneath the endothecium are middle layers, which are radially flattened. These ephemeral cells show signs of being crushed at the time of meiotic divisions in the microspore mother cells (Fig. 1G, H). During the further development of the anther, these layers get completely absorbed. The innermost layer is the tapetum and it is of the secretory type. In the initial stages, each tapetal cell shows a prominent nucleus and dense cytoplasm. Later, these cells undergo radial elongation and become binucleate at the time of meiotic divisions in the microspore mother cells (Fig. 1I). Soon after the formation of sporangial wall, the ground parenchyma of connective cells immediately internal to the microspore mother cells become differentiated. They grow rapidly and become internal continuation of the tapetum forming an uninterrupted layer completely surrounding the sporogenous tissue. This part of the tapetum can be designated as the connective tapetum (Fig. 1H). Thus the tapetum is dual in origin. These tapetal cells differ morphologically with the cells of the parietal tapetum by their greater radial elongation, increased vacuolation and in having two or more nuclei. Hence the tapetum is dual in origin and dimorphic in nature.

Along with wall layers, the sporogenous tissue also develops. These cells enlarge considerably and surpass the size of the wall cells. Primary sporogenous cells are limited in number and they directly act as the spore mother cells without undergoing any further divisions. These cells can be readily differentiated from the other cells by their larger size, conspicuous nuclei and dense cytoplasm (Fig. 1H). In the initial stages these cells are closely packed together and are radially elongated, but shortly before meiosis they appear almost spherical in their shape. The first meiotic division in these cells is not followed by cytokinesis. The meiotic divisions are synchronous in all the meiocytes of an anther locule, but not in the different locules of the same anther. Cytokinesis takes place by furrowing. Microspore tetrads are mostly tetrahedral in their arrangement (Fig. 1K).

The individual microspore is first pyramidal in shape, because of the contact

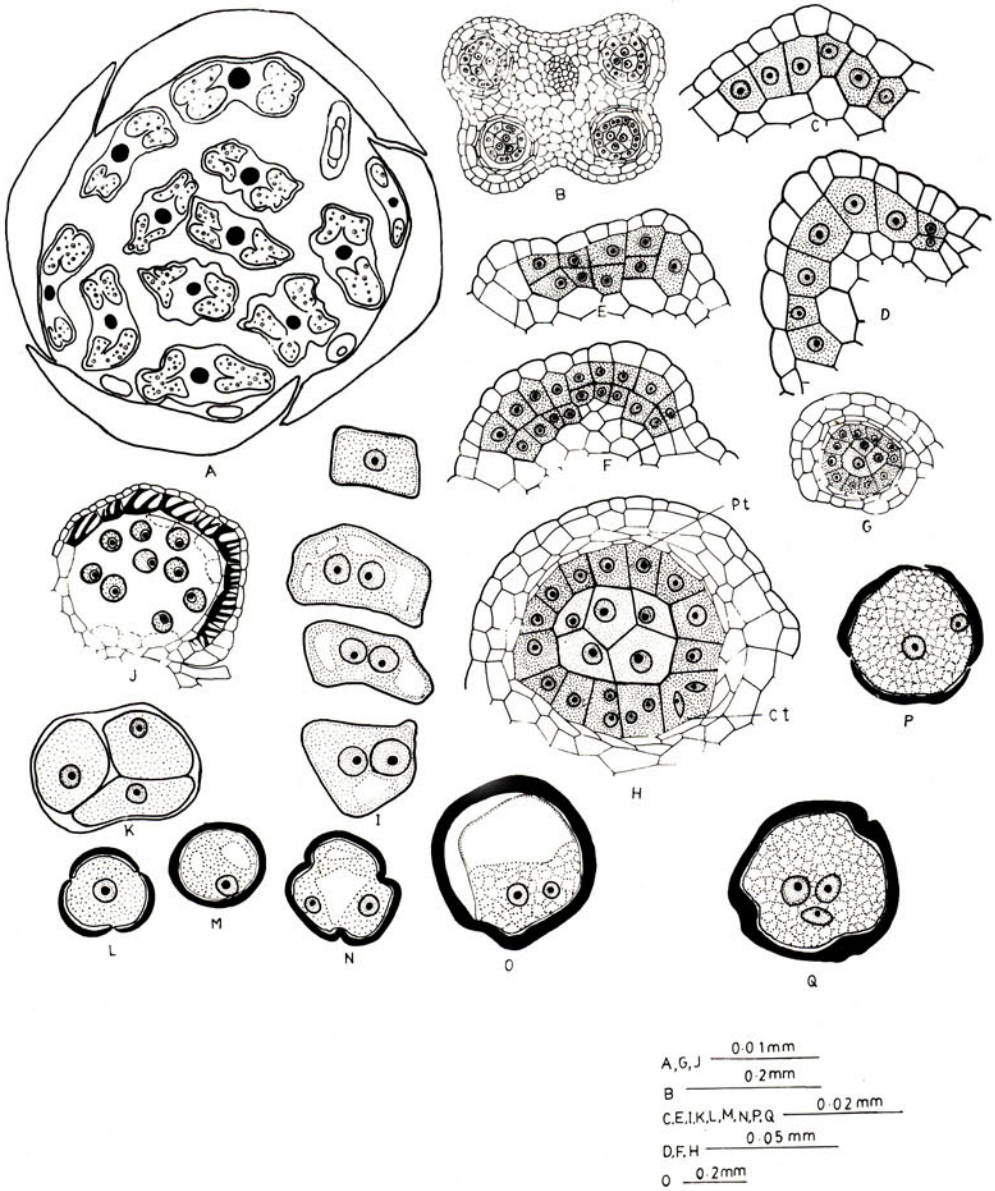

Fig. 1. A, C, E, G, L, M & N: *Diospyros chloroxylon* Roxb. B, D, F, H, I, J, K, O, P & Q: *D. virginiana* L.

A. T. s. of male flower showing 16 anthers.
B. T. s. of anther lobe showing tetrasporangiate nature.
C, D. T. s. of an young anther lobe showing archesporium.
E, F. T. s. of an young anther lobe showing primary parietal and primary sporogenous layers.
G, H. T. s. of anther lobe showing primary sporogenous cells, tapetum and wall layers. Note the connective tapetum (ct).
I. Tapetal cells with 1-2 nuclei.
J. T. s. of mature anther lobe showing well developed endothecium and pollen grains.
K. Microspore tetrad.
L. Pollen grains.

with each other in the tetrad. They rapidly enlarge and become nearly spherical and get liberated from the mucilagenous wall of the microspore mother cell. The newly formed microspore has a dense cytoplasm and the nucleus is centrally located (Fig. 1L). However, during further development, vacuoles arise and push the nucleus towards the wall (Fig. 1M), where the nucleus undergoes mitotic division, giving rise to a large vegetative and a small generative nuclei (Fig. 1N, O). The wall of the microspore becomes the wall of the male gametophyte. The pollen grains are 2-celled at the time of liberation (Fig. 1P). However, rarely some pollen grains show three nuclei in the vacuolated cytoplasm at the time of shedding (Fig. 1Q). The mature pollen grains are 3-colporate, prolate-spheroidal ($33\times25\,\mu m$) and psilate (Fig. 1N, Q). Pollen degenerations are quite common in *D. virginiana*. During the maturity of the anther a few cells of the epidermis at the junction of the two microsporangia become differentiated as the stomium. The cells of this region are thin walled and are slightly larger than the rest of the epidermal cells (Fig. 13). The anther dehiscence takes place at this point and the mature pollen grains are liberated.

DISCUSSION

The floral morphology of the members of the *Disospyros* studied at present is in agreement with the previously investigated members of the family. In the present study the anther is tetrasporangiate as in *Diospyros kaki* (Yasui, 1915). The anther wall development in the members studied at present is Dicotyledonous type and at maturity it consists of the epidermis, subepidermal fibrous endothecium, 1-2 middle layers and a secretory type of tapetum. In *D. kaki* (Yasui, 1915) the subepidermal layer did not develop any fibrous thickenings and remains non-fibrous. The tapetum (present study) to begin with, is uniseriate and the cells are uninucleate. But later, due to nuclear divisions the number of nuclei in each tapetal cell increases. Similarly in *D. kaki* (Yasui, 1915) the tapetal cells become multinucleate.

In the two species of *Diospyros* investigated at present a part of the tapetum is formed from the parietal layers and another part of it is derived from the connective cells and forms the connective tapetum (C-tapetum). Hence the tapetum is dual in its origin. The cells of the connective tapetum also differ slightly in their morphology by their larger size, increased vacuolation etc. from that of the parietal tapetum. Hence the tapetum can also said to be dimorphic in nature. Thus the tapetum in the members studied at present is dual in origin and dimorphic in nature. Such a tapetum was not reported in any other members of the family so far investigated. But a similer type of tapetum which is dual in origin and dimorphic in nature was reported by Periasamy and Swamy (1966), Vijayaraghavan and Ratnaparkhi (1973) and Lakshminarayana and Maheswari Devi (1985) in the members studied by them.

In the present study, the mature pollen grains are 2-celled at the time of liberation. But rarely, 3-nucleate pollen grains are also encountered in *D. virginiana*. Hague (1911) also reported a rare occurrence of the two celled pollen grains at the time of shedding in *D. virginiana* in his morphological study of the plant. One celled pollen grains of doubtful fertility were noticed in *D. kaki* (Yasui, 1915). The mature pollen grains are tricolporate, prolate-spheroidal and psilate and in agreement with the earlier palynological studies of Sharma and Gupta (1979).

The palynological studies have revealed that the family Ebenaceae is stenopalynous with a single morphological pollen type through out. The arrangement, position and the number of stamens show the primitiveness of the Ebenaceae among the Sympetalae (Yasui, 1915).

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兩種柿屬之小孢子囊及雄配子體的發育

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摘　　要

本文研究柿屬之綠材柿 (*Diospyros chloroxylon* Roxb.) 及美國柿 (*D. virginiana* L.) 的小孢子囊及雄配子體的發育。花藥壁的發育屬雙子植物型，成熟時含有表皮組織，纖維狀外層組織 (2～3 層) 及一單層之分泌型營養層，具單或雙核。結締營養層亦有分化。花粉母細胞之分裂爲同調並產生四同體之四分孢子細胞質分裂爲溝裂。花粉表面平滑，具三溝孔，由花藥釋出時爲雙核，很少有三核。在美國柿常見有花粉退化的情形。