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Studies in the Family *Ranunculaceae* — Microsporangium, Microsporogenesis and Ubisch Granules in *Nigella damascena*

By

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With 3 Figures

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Introduction

In the order *Ranales*, *Ranunculaceae* are the largest family comprising about 49 genera and about 500 species. The family is characterised for its remarkable combination of primitive and advanced characters viz. actinomorphic or zygomorphic flower; free or fused carpels; multiovulate follicles or uniovulate achenes; crassi- or tenuinucellar ovules which may be ategmic, unitegmic or bitegmic; persistent or ephemeral antipodal cells and occurrence of mono, bi, and tetrasporic embryo sac development. The genus *Nigella* is unique in exhibiting connation of carpels and an involucre of much dissected leaves beneath each flower. Although many taxa of this family have been investigated from the point of comparative embryology, information on *Nigella* is meagre. This is particularly so with regard to the microsporangium, microsporogenesis and male gametophyte. This prompted us to take up this present investigation on *Nigella damascena* L.

We are grateful to Professor B. M. Johri for encouragement and Dr. K. M. M. Dakshini for valuable suggestions.

Material and Methods

The material of *Nigella damascena* was collected from the University Botanical Gardens, Delhi by Dr. N. N. Bhandari and Mr. Karan Kaul. To them we extend our warmest thanks. Buds and flowers were fixed in FAA (formalin, 5 ml; acetic acid, 5 ml; 50% ethyl alcohol, 90 ml) and were subsequently stored in 70 per cent ethanol. Following the usual methods of dehydration, infiltration and embedding, the material was

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sectioned between 5 and 8 microns depending upon the stage of development and stained either with Safranin fast green or Haematoxylin fast green. Supplementary examination of the pollen mother cells was also made by acetocarmine squashes.

Observations

Microsporangium: The prearchesporial anther is oval in transection and consists of homogeneous mass of cells with a well defined epidermis and a poorly developed procambium (Fig. 1 A). As the anther enlarges four hypodermal archesporial cells, which are deeply stained as compared to the other cells, differentiate one in each lobe of anther (Fig. 1 B).

Each archesporial cell divides periclinally to form an outer primary parietal cell and an inner sporogenous cell. Following this, the primary sporogenous cell increases in size, while anticlinal divisions take place in parietal layers leading to a considerable difference in two types of cells. The anther wall, thus consists of three layers of cells. The primary sporogenous cell undergoes periclinal divisions to form secondary sporogenous cells which finally form the microspore mother cells. Along with the formation of microspore mother cells, primary parietal cells undergo periclinal divisions resulting in the formation of secondary parietal cells 1 and 2 (Fig. 1 C, G). The former (spl_1) divides periclinally forming two layers of cells, namely the endothecium and the upper middle layer, while the latter (spl_2) segments periclinally to form the lower middle layer and the tapetum (Fig. 1 D, H). The ontogeny of the five wall layers in relation to the development of sporogenous tissue is shown in the scheme.

The mature anther comprises five wall layers and no additional cell layers are formed (Fig. 1 E, F, I, J). The development of the anther wall follows the Basic Type (Davis 1966). Endothecial cells undergo anticlinal divisions, finally enlarge and develop characteristic band like thickenings (Fig. 2 A, D). The two middle layers are crushed but their nuclei can be recognized for a long time. The tapetal cells, soon after their formation,

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Fig. 1. *Nigella damascena*, Microsporangium (arc = archesporium, en = endothecium, ep = epidermis, ml_1 = middle layer 1, ml_2 = middle layer 2, ppl = primary parietal layer, Spl_1 = secondary parietal layer 1, Spl_2 = secondary parietal layer 2, spor = sporogenous cell, t = tapetum). — A: T. s. undifferentiated anther; ×304. — B: Transection of anther showing hypodermal archesporial cell and primary parietal cell; ×304. — C—F: Transverse section of anthers at different stages of development; ×40. — G: Magnified view of portion marked g in C, to show primary parietal cell and secondary parietal cells 1 and 2; ×304. — H: Enlarged view of sector marked *h* in D showing epidermis, endothecium, 2 middle layers; endothecium and upper middle layer are derivatives of Spl_1, whereas lower middle layer and tapetum are derived from Spl_2; ×304. — I, J: Magnified view of the sectors i and j marked in E and F respectively to show epidermis, endothecium, 2 middle layers and tapetum; ×304.

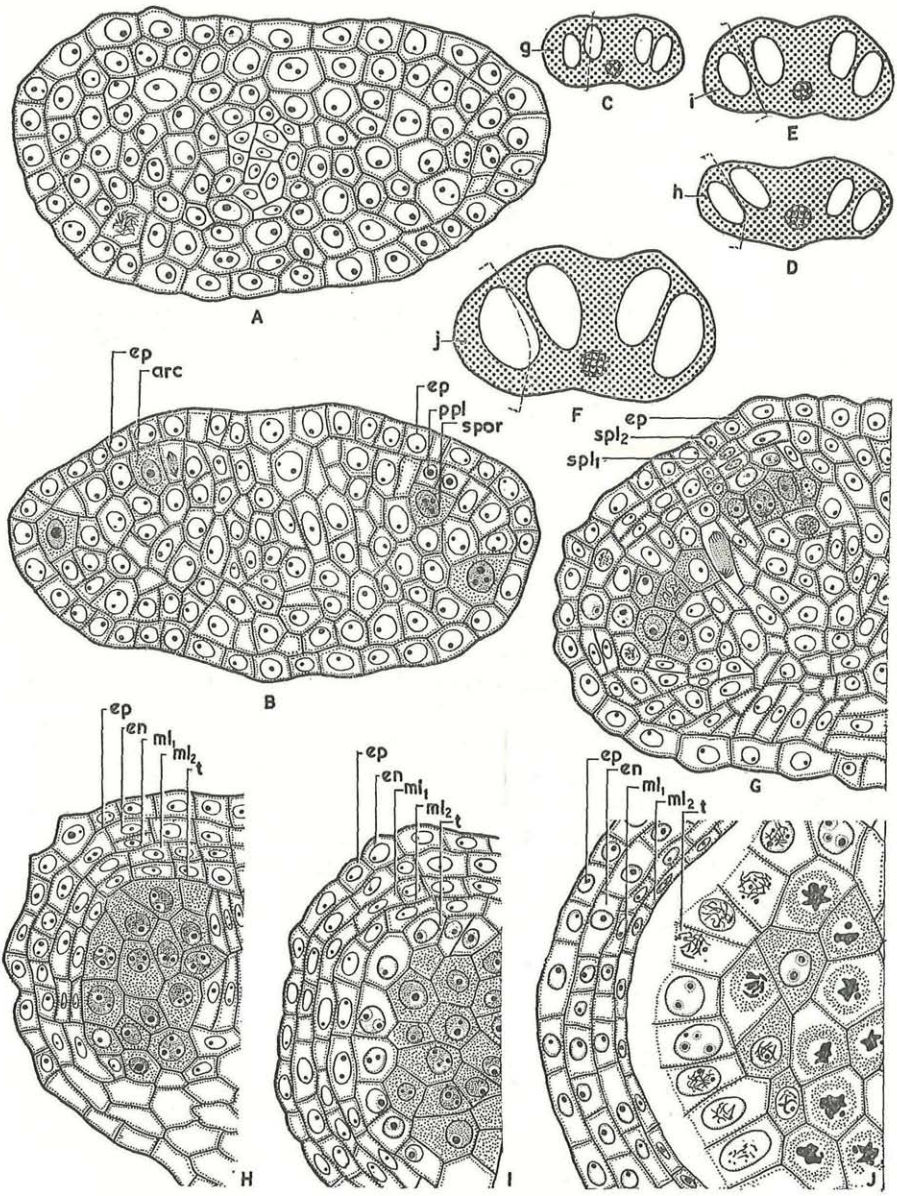
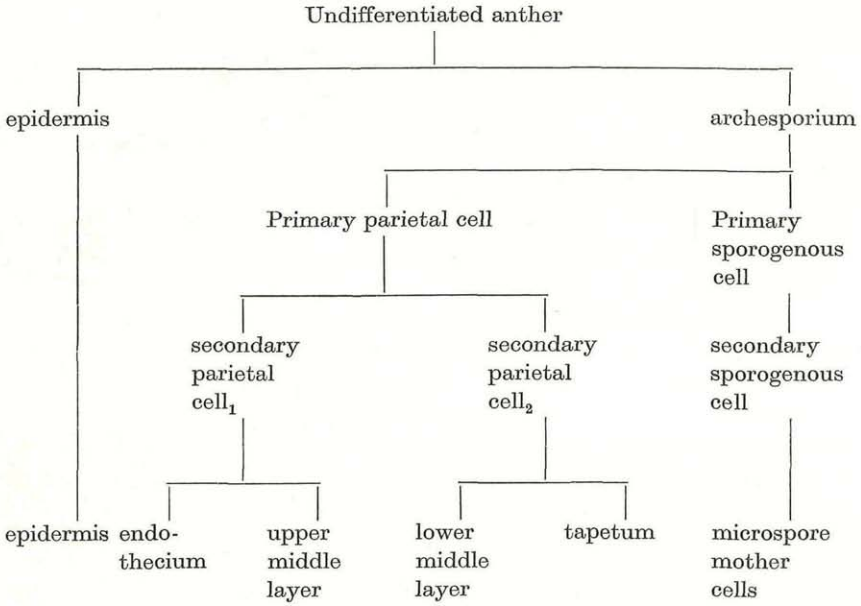


Fig. 1. For explanation see opposite page.



start elongating and attain maximum development during microsporogenesis. The tapetal cells are at first uninucleate but due to mitotic divisions become multinucleate and subsequently the nuclei fuse and become polyploid (Fig. 2 E—J).

Microsporogenesis and male gametophyte: Microspore mother cells undergo meiosis and cytokinesis is of the simultaneous type (Fig. 2 K—P). The microspore tetrads are tetrahedral (Fig. 2 Q) or decussate. The microspores soon after their liberation show typical tricolporate structure with thick exine and thin intine (Fig. 2 R). At the onset of gametogenesis the nucleus of the microspore moves toward one side and divides to form a large vegetative and a small generative cell. The pollen grains are shed at the 2-celled stage (Fig. 2 S).

Ubisch granules: At about the microspore tetrad stage Ubisch granules appear on the inner tangential and radial walls of tapetum. These granules are also present on the septa of the anther loculus (Fig. 3 A, B). During the uninucleate pollen grain stage the tapetum starts degenerating and by the time the pollen is 2-celled the tapetum is completely absent but the Ubisch granules are restricted only on the septum (Fig. 3 C, D).

Discussion

In *Nigella damascena*, anther wall comprises persistent epidermis, endothecium, two middle layers and multinucleate tapetum as in *Caltha palustris* and *Actaea spicata* (Jalan 1963). Davis 1966 reported Dico-

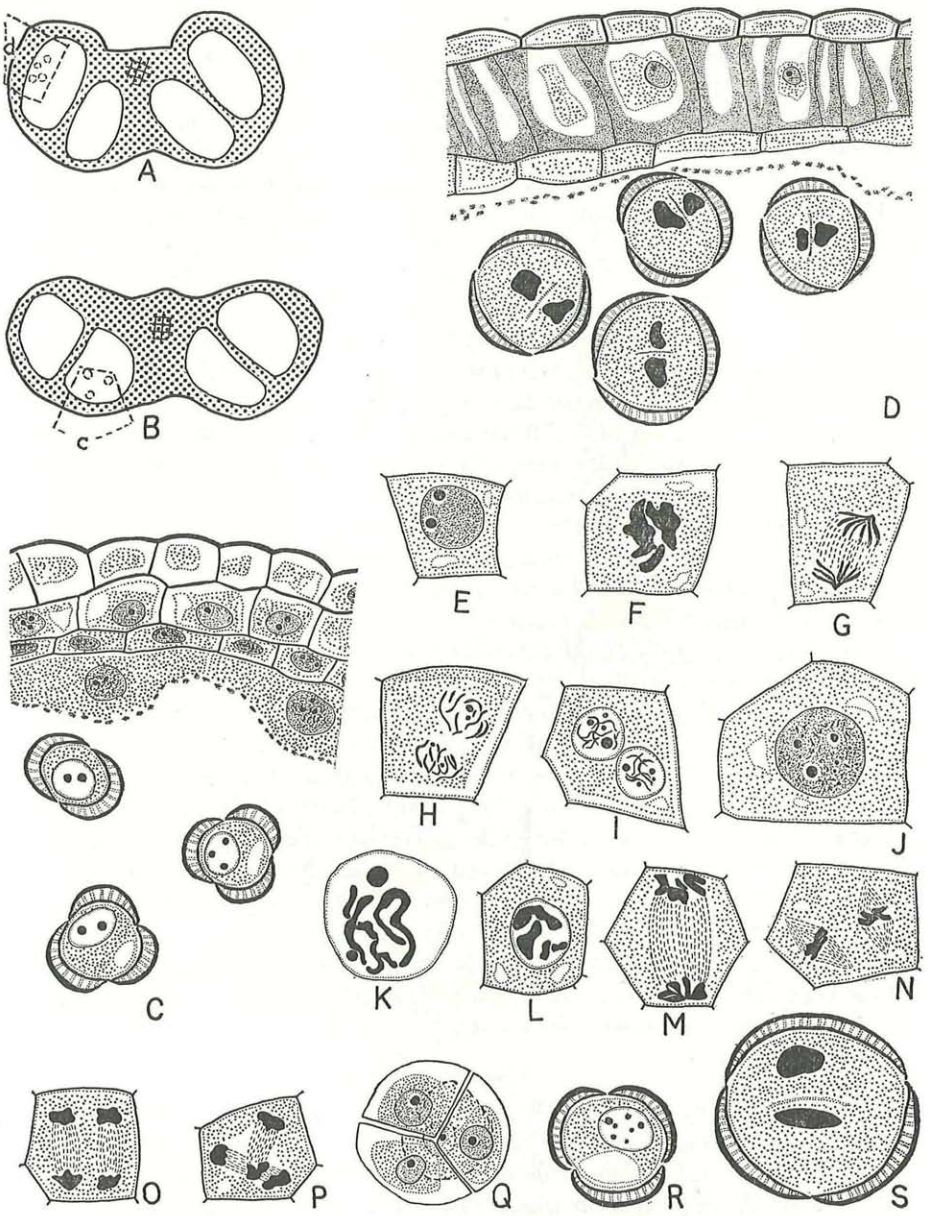


Fig. 2. *Nigella damascena*, Microsporogenesis. A, B: Transection of anthers at different stages of development; ×40. — C, D: Magnified view of the portions marked d and c in A and B respectively showing wall layers, one middle layer has already degenerated and the endothecium shows band like thickenings in D; ×304. — E—J: Nuclear behaviour in tapetal cells; ×760. — K—P: Meiosis in microspore mother cells; ×760. — Q: Tetrahedral tetrad; ×760. — R, S: One and 2-celled pollen grains; ×760.

tyledonous type of anther wall development for the *Ranunculaceae*. Contrary to this, the present work on *Nigella damascena* indicates that the wall development follows the Basic type and the same is true for *Ranunculus sceleratus* and *R. abortivus* (unpublished observations). The tapetal cells in *Delphinium ajacis* are uninucleate (COOPER 1933) whereas in *Hepatica acutiloba* as many as 13 nuclei have been reported (SCHNARF 1931). In *N. damascena*, there are 2 to 4 nuclei which later fuse to form polyploid mass. Similar behaviour of tapetal nuclei has also been reported in *Caltha palustris* (KAPIL & JALAN 1962), *Actaea spicata* (JALAN 1963) and *Clematis gouriana* (VIJAYARAGHAVAN 1963). JALAN 1963, observed in *A. spicata* amoeboid tapetum for the first time in this family. The meiotic divisions in the microspore mother cells of *N. damascena* are of the simultaneous type resulting in tetrahedral or decussate arrangement of tetrads as is found in many other genera of the family. The pollen grains are shed at the 2-celled stage in contrast to the 3-celled condition in *Myosurus minimus* (SWINGLE 1908).

In *Nigella damascena* prominent Ubisch granules are present on the inner tangential and radial walls of the tapetal cells. These granules are also met with on both the margins of the septa in the anther loculus. ROSONOFF 1865 (quoted in HESLOP-HARRISON 1963) was the first to observe these particles in the anther tapetum and referred them as 'tapetal plaques'. UBISCH 1927 and KOSMATH 1927 observed that these tapetal plaques behave like sporopollenin, the material of the pollen exine, in staining properties and in resistence to acetolysis. HESLOP-HARRISON 1962, has shown that these tapetal granules get deposited on the inner tangential and radial walls of the tapetal cells in *Silene pendula* at the early pollen tetrad stage. In *Nigella damascena* also, these granules stud the inner tangential and radial walls at about the same stage. Only a few Ubisch granules are present at the 2-celled stage of the pollen grain when the tapetum is completely degenerated. Further, they were also seen on both the margins of the septa in an anther loculus. At the time of dehiscence of anther, these granules were seen studded on the septal walls.

Summary

The ontogeny of the anther wall is described for the first time for *Nigella damascena* and it corresponds to the Basic type contrary to Dicotyledonous type for the *Ranunculaceae* (DAVIS 1966). Anther wall comprises the epidermis, endothecium whose cell acquire band-like thickenings, two ephemeral middle layers and a glandular tapetum. Ubisch granules not only stud the inner tangential and radial walls of the tapetum but also both margins of the septa in the anther loculus. Cytokinesis is of the simultaneous type and the microspore tetrads are either tetrahedral or decussate. Pollen grains are tricolporate, with thick exine and thin intine. They are shed at the two celled stage.

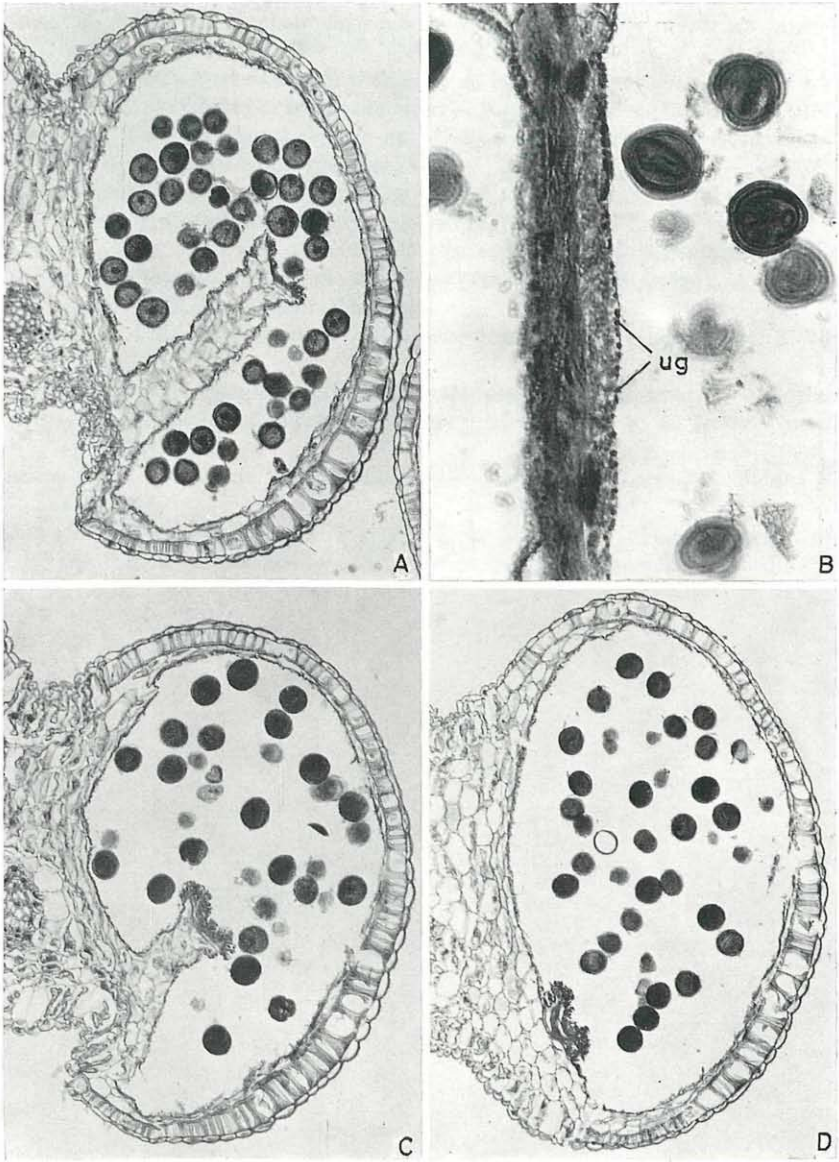


Fig. 3. *Nigella damascena* (ug = Ubisch granules). — A: T. s. anther lobe showing well developed pollen grains and Ubisch granules on the septa; ×540. — B: A portion of the septa in an anther loculus enlarged to show prominent Ubisch granules and well developed pollen grains; ×1632. — C, D: Later stages showing degeneration of anther septum, but a few Ubisch granules still stud the septum; ×410.

References

Cooper D. C. 1933. Nuclear divisions in the tapetal cells of certain angiosperms. — Amer. J. Bot. 20: 358—364.

Davis G. L. 1966. Systematic Embryology of the Angiosperms. — New York.

Heslop-Harrison J. 1962. Origin of exine. — Nature (London)195: 1069—1071.

— 1963. Ultrastructural aspects of differentiation in sporogenous tissue. — In: Symp. Expl. Biol. 18: 315—340.

Jalan S. 1963. Studies in the family *Ranunculaceae*-VI. The embryology of *Actaea spicata* Linn. — Phytomorphology 13: 338—347.

Kapil R. N. & Jalan S. 1962. Studies in the family *Ranunculaceae*-I. The embryology of *Caltha palustris* L. — The Plant Embryology. A Symposium, CSIR, New Delhi: 205—214.

Kosmath L. 1927. Studien über das Antherentapetum. — Österr. bot. Z. 76: 235—247.

Schnarf K. 1931. Vergleichende Embryologie der Angiospermen. — Berlin.

Swingle L. D. 1908. Embryology of *Myosurus minimus* L. — Amer. Natur. 42: 582—591.

Ubisch G. V. 1927. Zur Entwicklungsgeschichte der Antheren. — Planta 3: 490—495.

Vijayaraghavan M. R. 1963. Morphological and Embryological Studies on the families *Actinidiaceae, Chloranthaceae* and *Ranunculaceae*. — Ph. D. Thesis, Univ. Delhi.