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Male and Female Gametophyte Development of *Seseli resinosum* (Apiaceae)

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Abstract

The male and female gametophyte development in *Seseli resinosum* Freyn et Sint were studied with alight microscope. Anther wall development follows the dicotyledonous type: epidermis, endothecium, middle layer, and tapetum. The tapetum is secretory andthe tapetal cells are two-, three- and four-nucleate. The microspore tetrads are tetrahedral and isobilateral,and pollen grains are shed during a three cellular stage. The mature ovule is anatropous, tenuinucellar and unitegmic. A funicular obturator is present, and the hypostase is absent. The nucellarepidermis is one layered.Absorption of the nucellus occurs in four-nucleate embryo sac. A linear megaspore tetrad is formed, and the megaspore on the chalazal side remains active. The functional megaspore produces a seven-celled embryosac corresponding to the Polygonumtype. The embryo sac formation is monosporic.

Keywords: *Seseli resinosum*, Apiaceae, female gametophyte, male gametophyte

INTRODUCTION

The genus *Seseli* L., which belongs to the Apiaceae family,has economic importance. Dried base leaves are used as animal food, and the plant itself is used an ornamental plant in gardens and parks. *Seseli* chemicals are used in medicine [1-4].

Seseli resinosum Freyn et Sint is an endemic species that is widely distributed in the Western Black Sea region of Turkey [5-6]. The environments that harbor the species are quite healthy. However, both the province of Zonguldak and tourist destinations withtheir sprawling communities represent adanger to which the species is vulnerable (VU) [7].

The author's previous study of the Apiaceaespecies , including *Aegopodium podagraria L., Bupleurum mucronatum Wight et Arn., Cuminum cyminum L., Coriandrum sativum L., Daucus carota L., Daucus muricatus L., Eryngium yuccifolium Michx., Ferula sinkiangensis KM. Shen*, *Foeniculum vulgare* Mill., *Hydrocotyle americana L.*, *Osmorhiza longistylis*(Torr.) DC., *Pastica sativa* L., *Pimpinella diversifolia* D.C., *P. candolleana*Wight etArn., *P. heyneana* Wall., *P. bracteata* Haines, *P. monoica*Dallz., *Trachyspermum ammi L. (Sprague), and Zizia aurea (L.)* W.D.J. Koch.,revealed that the embryogeny is *Solanad* type and that the embryo development is *Polygonum* type.The embryo sac may be mono-, bi-, or tetrasporic, although the first type is predominant [8,24]

The endosperm is the nuclear type in all the plants studied [20,23,24].

The pollen morphology of the Turkish *Seseli* L. species was studied by Doğan Güner et. al.[4]. Based on our search of the literature,the male and female gametophyte characteristics of *S. resinosum* have not been previously studied.

The purpose of this work is a detailed investigation of the female and male gametophytedevelopment of *S. resinosum*.

MATERIALS and METHODS

In July and August of 2007-2009, 300 flower buds and 100 flowers inbloomwere collected from plants that grew in the rocky fields ofBartın-İnkumu and Zonguldak (Figure 1A). The *Seseli resinosum* flower stems from compound umbels and hermaphrodite. The ovary is inferior, bicarpellary, syncarpous and bilocular. There is one ovule in each locule (Figure 1B), which is surmounted by an epigynous disc commonly known as the stylopodium. The embryosac and pollen development were studied with light microscopy.

Ovules and anthers in different stages of development were fixed in formaldehyde, acetic acid, ethanol solution (FAA, 5:5:90), stored in 70% ethanol, embedded in paraffin, serially sectioned (7-8µm thick) with a Thermo-Shandon Finesse 325 rotary microtome and stained with hematoxylin [25-29]. For each male and female gametophyte development stage,150 flower buds were observed and measured. Because the development patterns examined in buds were generally small, bud lengths were measured on longitudinal sections. The mean and standard deviation were calculated using Microsoft Excel 2007.The lengt of the flower bud measurements were given in Table 1. The smallest bud was 0.35mm,the biggest bud was1.52 mm. The average length of blooming flowers was 2.23 ± 0. 74 mm.

Photomicrographs taken with a Nicon Eclips 200 and a Leica DFC microscope. Photomicrographs of the female gametophyte development pattern were arranged chalazalendbeing on the upper side.

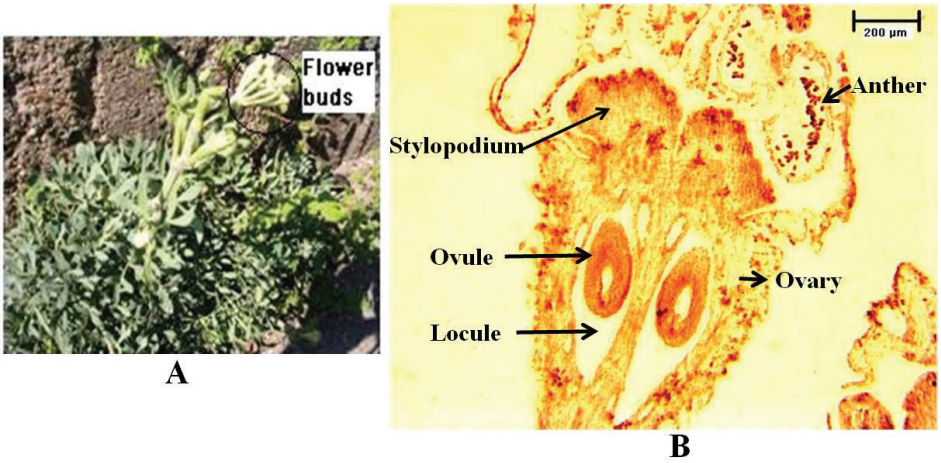


Figure 1. A. Photograph of the upper stem of *Seseliresinosum* showing flower buds. B. Longitudinal section of the flower bud.

Table 1. The flower bud length dimensions (mm) according to male and female gametophyte development patterns.

Male gametophyte Stages	Mean	Standard Deviation	Minimum	Maximum
Young Anther	0.83	0.15	0.58	1.10
Prophase I	0.93	0.20	0.64	1.43
Meiosis I	1.01	0.39	0.41	1.52
Metaphase I	0.98	0.27	0.75	1.25
Telophase I	0.81	0.34	0.41	1.25
Meiosis II	1.08	0.12	0.92	1.18
Telophase II	0.97	0.16	0.63	1.18
Simmultane sitokinesis	1.19	0.09	1.04	1.29
Tetrad	1.10	0.23	0.67	1.52
Microspore	1.12	0.23	0.66	1.35
One nuclear pollen grain	1.09	0.23	0.35	1.35
Two-celled pollen grain	1.50	0.49	0.92	3.71
Three-celled mature pollen grain	1.88	1.22	1.16	3.71
Female gametophyte stages				
Megaspore mother cell, unitegmic tenuinucellate ovule	0.90	0.21	0.41	1.08
Megasorogenezis Diad	1.26	0.27	0.99	1.52
The linear megaspore Tetrad	1.23	0.01	1.22	1.23
Faal megaspore	1.12	0.15	0.88	1.33
One nuclear embryo sac	1.68	0.00	1.68	1.68
2 nuclear emb.sac	1.29	0.12	1.19	1.47
4 nuclear embyo sac	1.25	0.13	0.99	1.34
8 nuclear mature embryo sac	1.67	0.56	1.26	3.71
Fertilization of egg cell	1.23	0.00	1.23	1.23
Zigote	1.99	0.09	1.89	2.08

RESULTS

Microsporangium

The anther contains four sporangia (Figure 2A). Differentiations between primary parietal cells can be observed(Figure 2 B-C). The microsporangial wall follows the dicotyledone type ontogeny [30,31]. The anther wall consists of an epidermis, endothecium, one middle layer, and a secretory tapetum (Figure 2D). The tapetal cells are generally binuclear,and three and four nuclearcells are rare (Figure 3 A-D).Some cells have nuclear fusions and dense cytoplasm (Figure3 A-D). During the maturation of the anther,endothecial cells thicken into fibrous bands in the radial walls (Figure4 A-B).

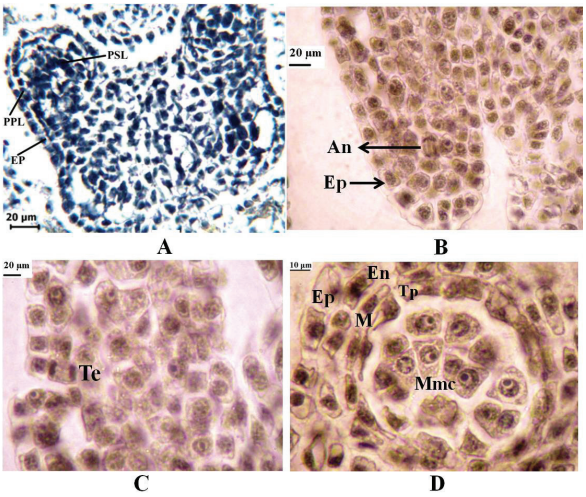


Figure 2. A. Cross section of a young anther. (B-C). Differentiation of wall layers in longitudinal sections of an anther. D. Four-layered young anther, An: Anaphase, EP, Ep: Epidermis, En: Endothecium, M: Middle layer, Tp: Secretory tapetum, Mmc: Microspore mother cell, PPL: Primary parietal layer, PSL: Primary sporogenous layer, T:Telophase.

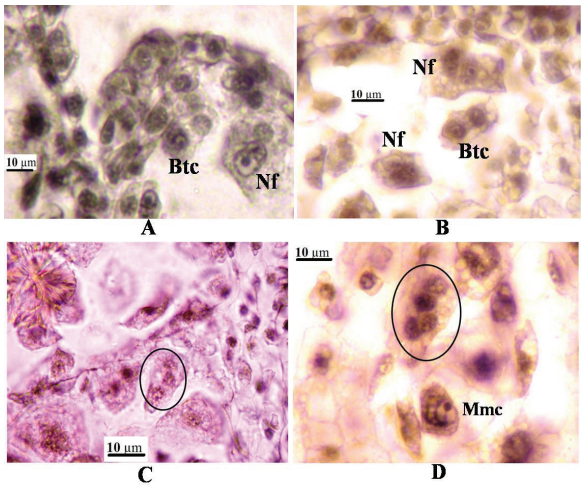


Figure 3.(A-B). Binuclear tapetum cells and nuclear fusions. C. Three nuclear tapetum cells. D. Four nuclear tapetum cells,Btc: Binuclear tapetum cells, Nf: Nuclear fusion, Mmc: Microspore mother cell.

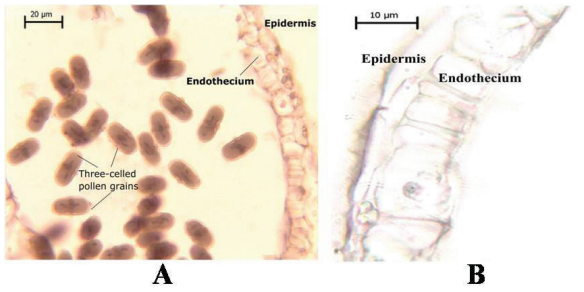


Figure 4.(A-B). Cross sections of a mature anther.

Microsporogenesis and microgametogenesis

The stages of meiosis in the microspore mother cell and microspore formation, as well as young and mature pollen, were observed in the flower bud cross-sections. Four stages of Prophase I (leptotene, zygotene, pachytene, diplotene) and all phases of meiosis could be distinguished (Figure 5A-F, Figure 6A-C).

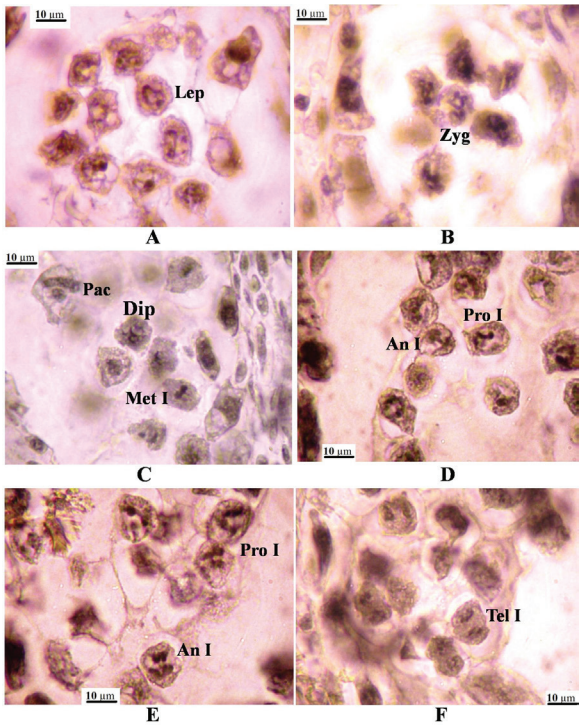


Figure 5.(A-F). Stages of Meiosis I in cross sections of young anthers. (A-E). Prophase. C. Metaphase. D. Abnormal Anaphase. E. Normal anaphase. F. Telophase. An: Anaphase I, Dip: Diplotene, Lep: Leptotene, Met I: Metaphase I, Pac: Pachytene, Tel I: Telophase I, Zyg: Zygotene.

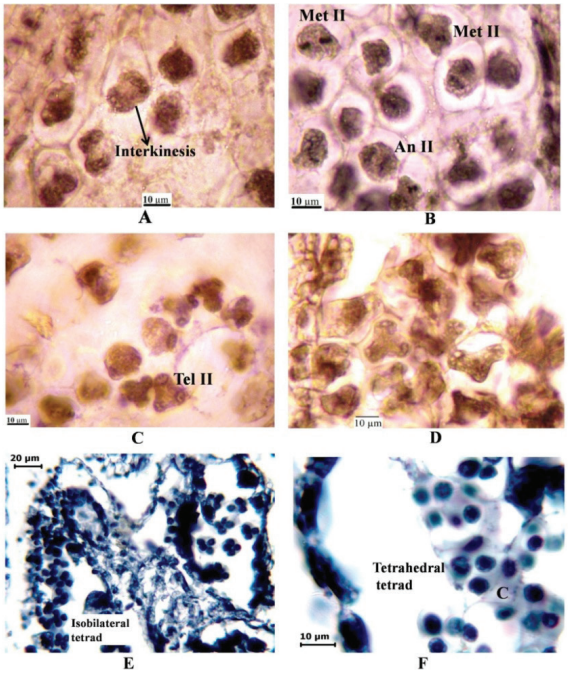


Figure 6. Meiosis II. A. Interkinesis. B. Metaphase II, Anaphase II. C. Telophase II. D. Simultaneous cytokinesis. E. Isobilateral. F. Tetrahedral Tetrad.

Cytokinesis is simultaneous, and the microspore arrangement is tetrahedral and isobilateral(Figure6 D-F). The microspore tetrads remain within the callosic walls during the late tetrad stage (Figure 6F).After meiosis, the peripherycallose dissolves and four microspores are released (Figure 7A).The microspores enlarge, and a central vacuole appears, pushing the nucleus toward the periphery. The tapetum degeneration is visible (Figure7B). As a result, the mitotic division of the microspore is unequal. Therefore, a small generative cell and a large vegetative cell appear(Figure7C). The first cell is separated from the wall of the pollen grain and lies in the cytoplasm of the vegetative cell (Figure 7C).The generative cell undergoes a second mitosis, and two sperm cells occur. Pollen grains are three-celled at the time of shedding (Figure7D). The tapetumcompletely degenerates during this process.The sperm cells acquirea fusiform shape in the last stage of development (Figure7D).

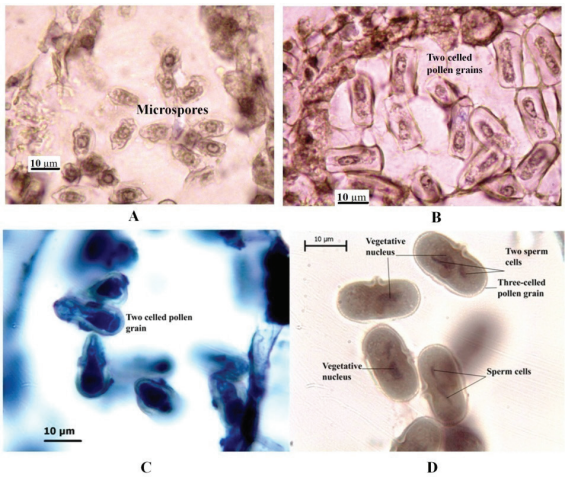


Figure 7.(A). Microspores.(B-C). Two-celled pollen grains.(D). Three-celled pollen grains.

Ovule

The mature ovule is anatropous, tenuinucellar, and unitegmic (Figure8A). The nucellar epidermis is present and persistent until the four-nucleate embryo sac stage. The integument is seven cells thick (Figure 8A).

The inner epidermis of the integument differentiates into an endothelium. There is a multicellular archesporium, but only one of the cells develops into a megaspore mother cell. The funicular obturator is present,and the hypostase is absent(Figure8 B-C).

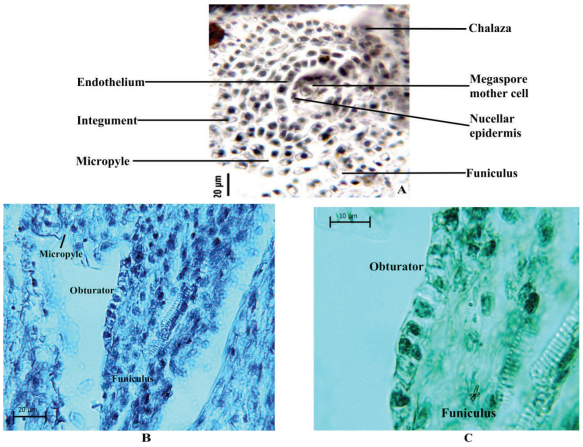


Figure 8.(A). Unitegmic and tenuinucellar ovule.(B-C). Funicular obturator.

Megasporogenesis and the female gametophyte

The megaspore mother cell (MMC) divides meiotically and undergoes two successive divisions, resulting in a linear tetrad (Figure9A-E). The three micropylar megaspores degenerate and the chalazal megaspore develops into the megagametophyte (Figure 9F-I).

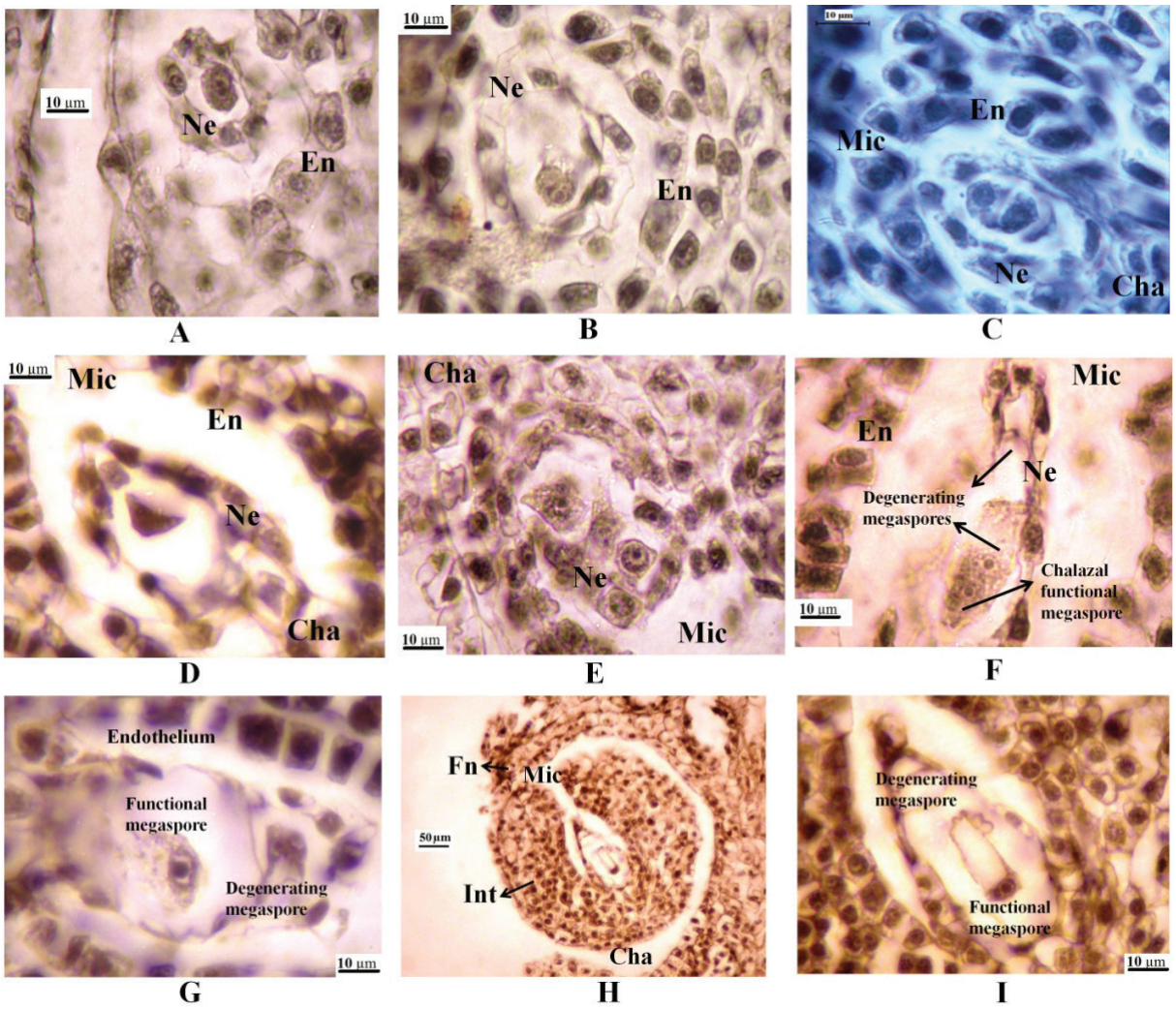


Figure 9. Megasporogenesis. (A-B-C). Dyad, D. Meiosis II. E. Linear tetrad.(F-G-H-I). Functional and degenerating megaspores, Cha: Chalaza, En: Endothelium, Fn: Funiculus, Int: Integument, Mic: Micropyle, Ne: Nucellar epidermis.

The functional megaspore elongates and moves to the center of the ovule (Figure 10A). The centrally situated nucleus divides mitotically to form two nuclei (Figure 10 B-C). The second mitotic karyokinesis gives rise to four nuclei that have across-shaped organization (Figure 10 D-E). The one-layered nucellus is greatly reduced and almost completely absorbed by the time the four-nucleate embryo sac is formed. During the subsequent development,the four-nucleate embryo sacis directly surrounded by the endothelium. After the third mitosis, the seven-nucleate embryo sac occurs.

The mature embryosac consists of seven cells: the egg cell, two synergids, the central cell or secondary nucleus and three very small antipodal cells (Figure11 A-C). This embryosac represents the *Polygonum* type. The egg cell and two synergids a lign at the micropylar end of the embryo sac.The synergids surround the egg cell (Figure 11D).The synergid pairs do not differ in size. The polar nuclei are almost the same size and fuse before fertilization to form a secondary nucleus near the egg apparatus(Figure 11E).

Fertilization

The pollen tube enters the embryo sac through the micropyle, and fertilization presumably takes place in the normal fashion (Figure 12A-C). The egg cell is 10.36 µm, and the sperm cell is 7.01 µm in width.

DISCUSSION

Our findingsshow some similarities between the embryology of the studied Apiaceae species.

The flowers of *Seseli resinosum* are minute, hermaphrodite, and borne in compound umbels. The anther wall comprises the epidermis, fibrous endothecium, single middle layer and secretory tapetum. The tapetal cells are two-, three-, and four-nucleate. The reduction divisions in the pollen mother cells are the simultaneous type and the tetrads are tetrahedral and isobilateral. The pollen grains are shed during the threecelled stage.These results are similar to *Cuminum cyminum*, *Trachyspermum ammi* [20], *Pimpinella diversifolia, P. candolleana, P. heyneana, P. bracteata, P. monoica* [22], *Bupleurum mucronatum*[23], *Ferula sinkiangensis* [24].However, three and four nuclear tapetal cells have never been reported before in the Apiaceae family.

The *Seseli resinosum* ovules are anatropous, unitegmic, and tenuinucellate. The single- layered nucellus persists during the four nucleate gametophyte stage. The endothelium and a funicular obturator are present,and the hypostase is absent. These results also have been found in Bubleurum mucronatum, Cuminum cyminum, Ferula sinkiangensis, Pimpinella spp. [20, 22,24].

The megaspore mother cell undergoes reduction divisions and forms linear tetrads. The development of the

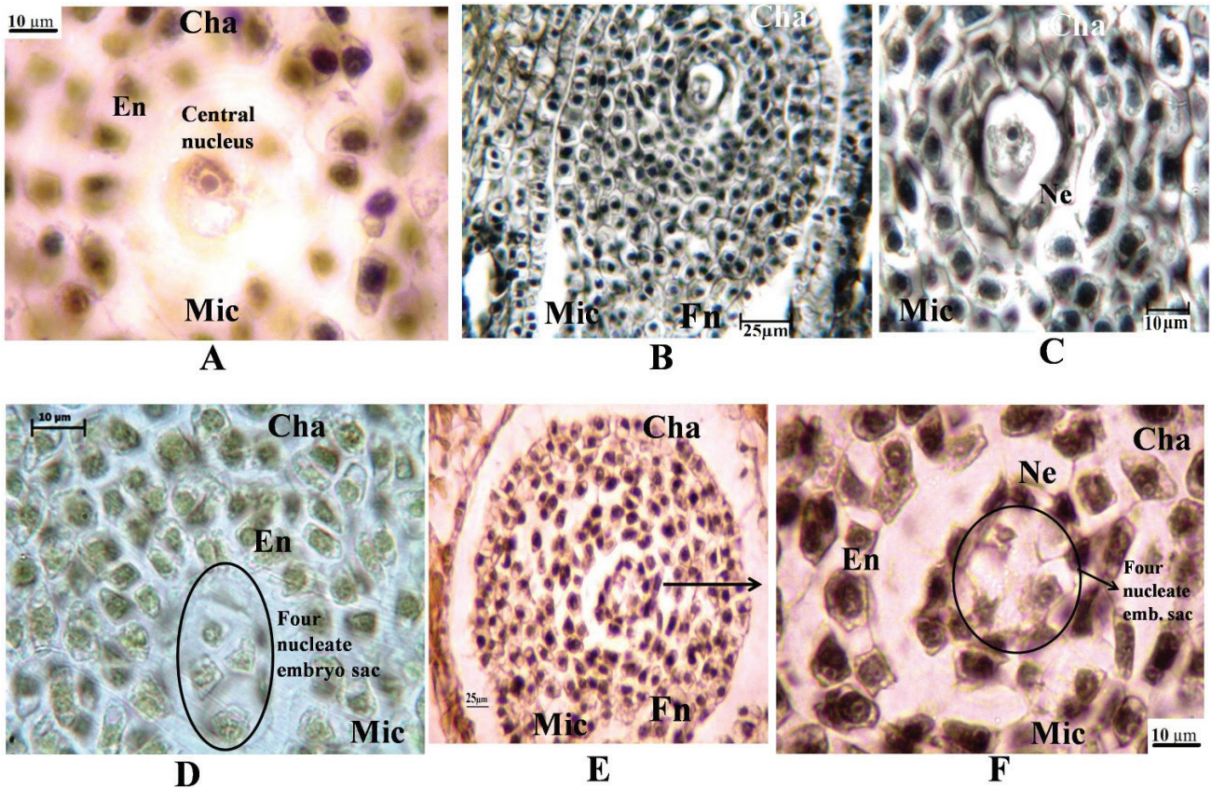


Figure 10. (A-E). Polygonum type embryo sac development. A. Centrally situated nucleus in cross section.(B-C). Two-nucleate embryo sac in longitudinal section.(D-E-F). Four-nucleate (two at the poles, two at the center) embryo sac in longitudinal section, Cha: Chalaza, En: Endothelium, Fn: Funiculus, Mic: Micropyle, Ne: Nucellar epidermis.

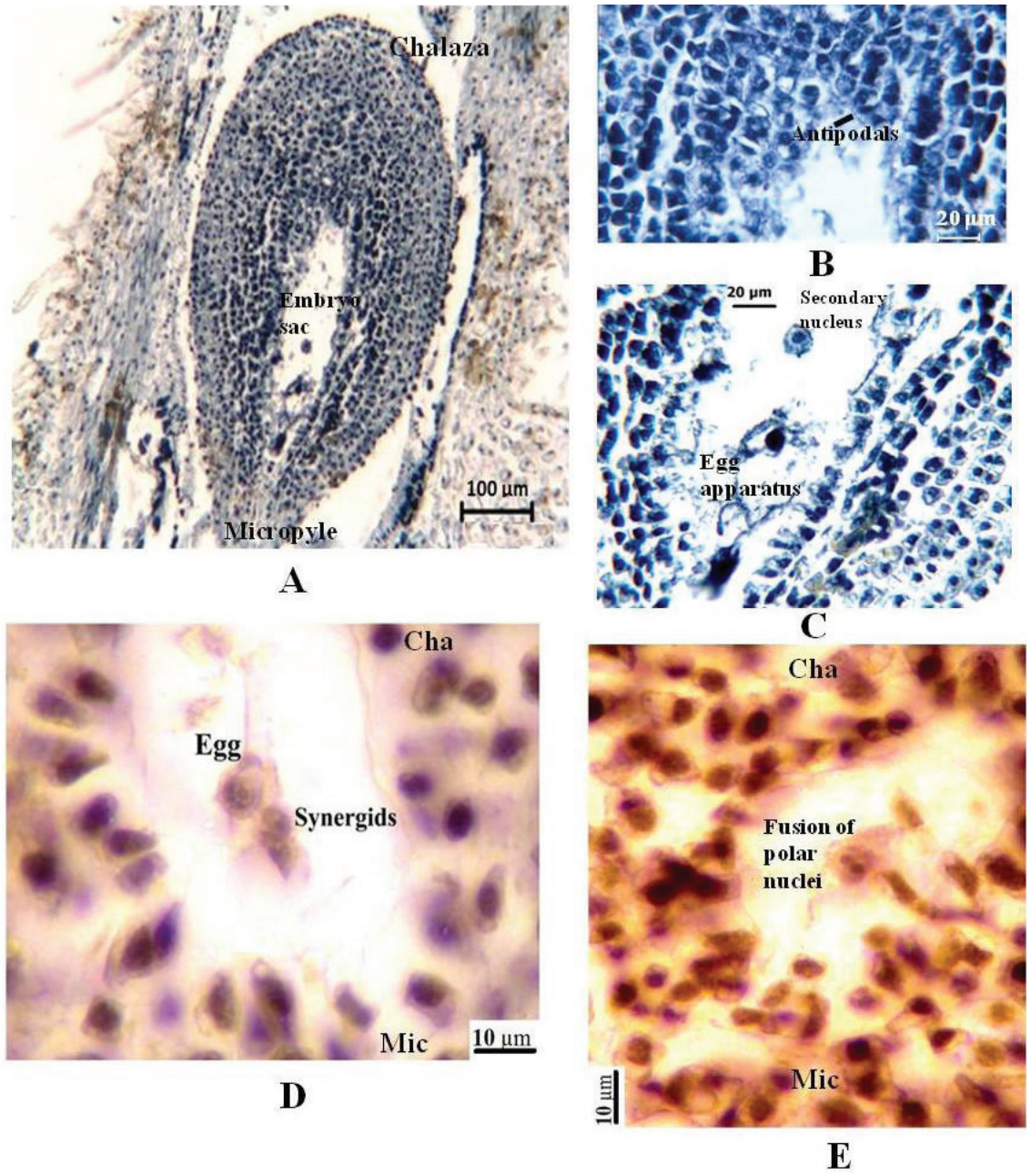


Figure 11. Longitudinal sections of ovules. (A-C). Mature embryo sac. (D). Egg apparatus. (E).Polar nucleus fusion,Cha: Chalaza, Mic: Micropyle.

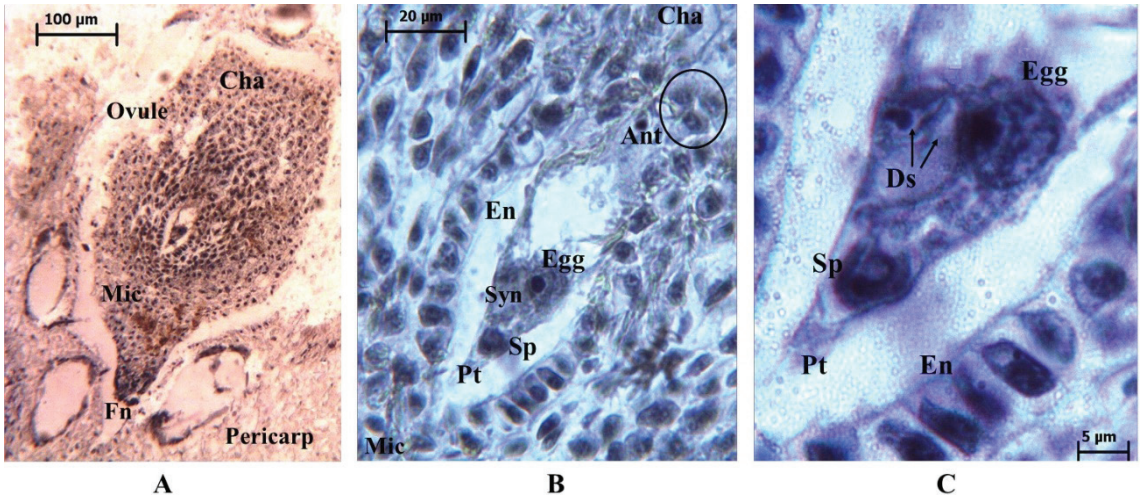


Figure 12. Fertilization of the egg cell. A. General view. (B-C). Degeneration of one synergid, Ant: Antipodals, Cha: Chalaza, Ds: Degenerated synergids, En: Endothelium, Fn: Funiculus, Mic: Micropyle,Pt: Pollen tube, Sp: Sperm cell, Syn: Synergids.

embryosac conforms to the *Polygonum* type. The organized gametophyte contains the egg apparatus, one secondary nucleus and three antipodal cells.

The male and female gametophyte development of *Seseli resinosum* shows some characteristics similar to that of *Bubleurum mucronatum*, *Cuminum cyminum, Ferula sinkiangensis, Pimpinella spp.,* and *Trachyspermum ammi*.

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