

## DEVELOPMENT OF MALE GAMETOPHYTE AND ORIGIN OF TAPETUM IN *SCUTELLARIA DISCOLOR* WALL EX. BENTHAM

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A perusal of literature reveals *Scutellaria discolor* remains uninvestigated. The anther tapetum, in a majority of the plants, develops from the inner parietal layer of the anther lobe, forming a homogeneous layer around the microsporangium<sup>1</sup>. However, in a few species with dimorphic tapetum, it has been suggested that the inner part of the tapetum develops from the adjacent cells of the connective, while the outer tapetum is formed from the inner parietal layer<sup>2,3</sup>. Hence, the structure, development and differentiation of male gametophyte and dimorphic tapetum in *S. discolor* were studied.

The young buds and flowers of *S. discolor* were collected from Western Ghats in Kodagu District, and fixed in formalin acetic acid alcohol (FAA). Customary methods of dehydration, infiltration and embedding were followed. Sections between 8 and 10  $\mu\text{m}$  were cut and stained in Heidenhein's iron-alum haematoxylin with erythrosin as the counter stain.

The young anther is four-lobed with a microsporangium in each lobe (figure 1). A plate of 6-8 hypodermal archesporial cells differentiates in each microsporangium (figure 2). As the development proceeds, each archesporial cell divides periclinally to produce primary parietal cell to the exterior and

primary sporogenous cell to the interior (figure 3). A periclinal division in the primary parietal layer results in the formation of outer and inner parietal layers (figure 4). The outer parietal layer divides periclinally to form endothecium and middle layer. The inner parietal layer remains undivided and acts as the presumptive tapetum. A row of connective cells adjacent to sporogenous tissue, meanwhile, differentiate into the tapetum (figures 4 and 5). The undivided inner parietal layer with isodiametric cells differentiates as outer part of the tapetum whereas the elongate, vacuolate, cells of the connective tissue adjacent to the inner face of sporogenous cells differentiate as inner part of the tapetum (figures 5 and 6). The binucleate tapetum persists for a long time till the formation of tetrads (figure 7), and degenerates prior to the anther dehiscence (figures 8 and 9).

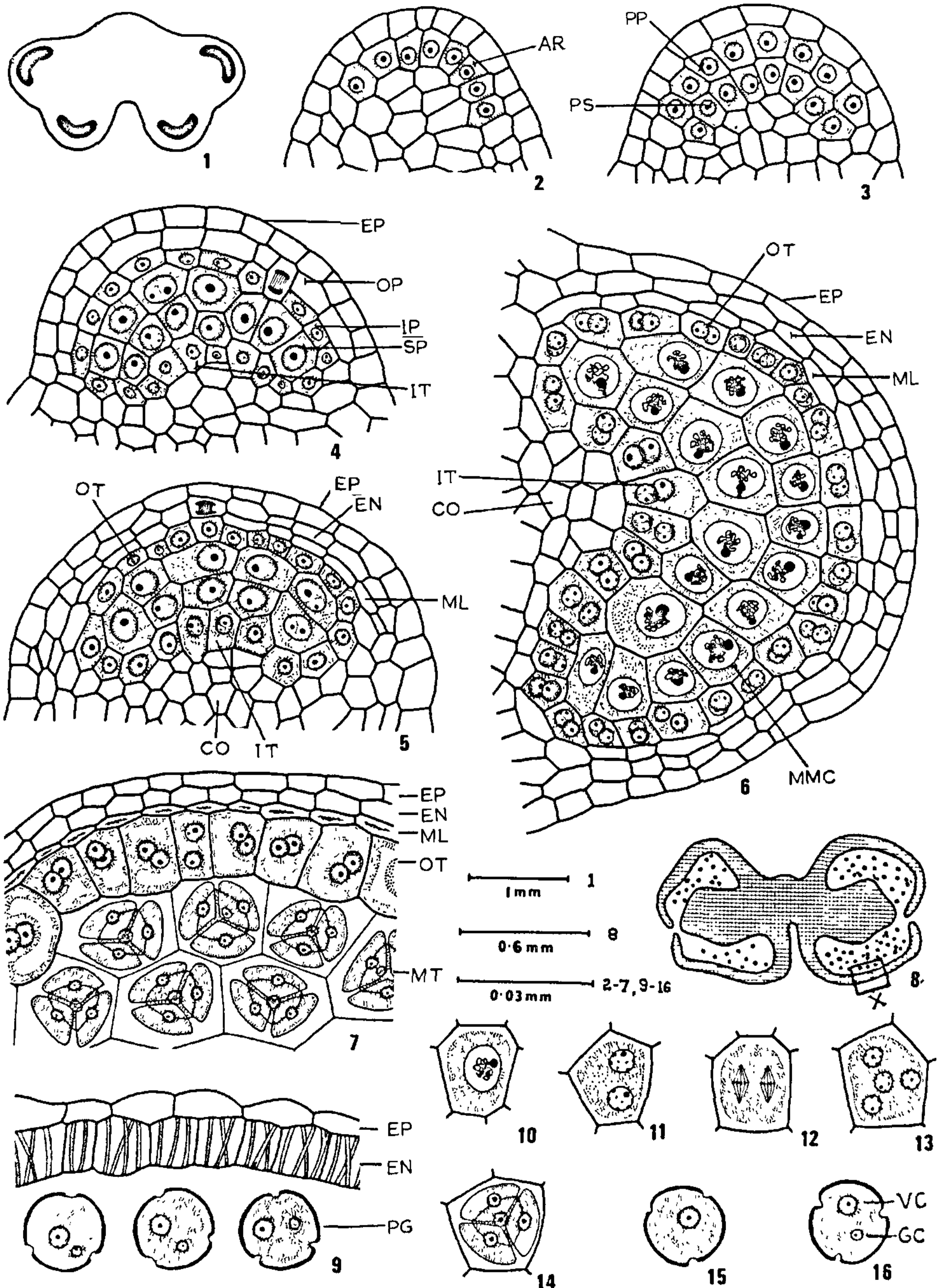
The cells of the primary sporogenous layer by several mitotic divisions give rise to a mass of sporogenous cells which ultimately differentiate into microspore mother cells (figures 5, 6 and 10). The microspore mother cells divide meiotically to form tetrahedral tetrads (figures 10-14). The individual microspores liberated from the spore tetrads enlarge in size and become spherical (figure 15). The microspore nucleus divides mitotically and form a small lenticular generative cell which soon enters into cytoplasm of large vegetative cell (figure 16).

The development of tapetal layer shows a deviation from the normal course, having a part of tapetum derived from parietal layer and the remaining part derived from the contiguous cells of the connective as reported in *Alectra thomsoni*<sup>3</sup>.

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**Figures 1-16.** 1. Transverse section of a young anther showing four microsporangia; 2. T. S. of microsporangium showing archesporial layer; 3-5. Same, showing primary parietal and primary sporogenous layer; note the division of the primary parietal cell; note the division in outer parietal layer; 6. T. S. microsporangium showing microspore mother cell and binucleate tapetum; 7. T. S. part of microsporangium showing degenerating middle layer and microspore tetrad; 8, 9. Outline of T. S. of mature anther showing the confluence of adjacent microsporangia and dehiscence; portion marked X enlarged, showing epidermis, fibrillar endothecium and bicelled pollen grains; 10-14. Formation of tetrahedral tetrads; 15. A microspore; 16. Two-celled pollen grain, (AR-archesporial layer, PP-primary parietal layer, PS-primary sporogenous layer, OP-outer parietal layer, IP-inner parietal layer, SP-sporogenous layer, EP-epidermis, EN-endothecium, ML-middle layer, OT-outer tapetum, IT-inner tapetum, MMC-microspore mother cell, MT-microspore tetrads, CO-connective region, PG-pollen grain, GC-generative cell, VC-vegetative cell).



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### TOXIN PRODUCTION BY *ALTERNARIA ALTERNATA* PATHOGENIC TO BRINJAL (*SOLANUM MELONGENA* L.)

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*SOLANUM MELONGENA* L. was found to develop a severe leaf spot disease during the rainy season of 1986. The pathogen was identified as *Alternaria alternata* (Fr.) Keissler based on the characteristics given by Ellis<sup>1</sup>. As the toxins produced by this pathogen play an important role in disease development<sup>2,3</sup>, an attempt was made to test the toxigenic potential of *A. alternata* pathogenic to brinjal.

The fungus was cultured on Czapek-Dox broth for 30 days as stationary culture at room temperature ( $28 \pm 4^\circ\text{C}$ ). The toxicity of the culture filtrate was tested on seed germination and root elongation of tomato at different dilutions (1:0, 1:2, 1:4, 1:8, 1:10, 1:20, 1:40, 1:60, 1:80 and 1:100 of culture filtrate and distilled water). The petri dishes containing the seeds treated with the culture filtrates were incubated for 96 h and the germination percentage and root length were recorded. The detached leaves of brinjal and tomato were transferred to test tubes containing culture filtrate and symptoms were observed after 24 h. Chloroform extracts of the culture filtrates were employed for testing the antibacterial activity against *Bacillus megaterium* and *B. subtilis* using the seeded plate method<sup>4</sup>.

The culture filtrates of the fungus grown on yeast-extract-sucrose broth for 30 days were screened for toxins<sup>5-7</sup>. The developing solvent system employed for TLC analysis was toluene-ethyl acetate-90% formic acid (5:4:1)<sup>8</sup>. On the basis of  $R_f$  values and fluorescence of spots, selected toxins were cochromatographed with authentic samples of *Alternaria* toxins and confirmatory tests were made<sup>5,9</sup>. UV spectral characteristics of the toxins were determined employing ethanol or water as carrier solvents<sup>10</sup>.

The reduction in seed germination and root length over control was as high as 79% and 87% respectively. At lower dilutions only, the toxic principle was inhibitory to tomato, but at higher dilutions it stimulated the seed germination and root elongation. Epinasty with inward rolling of the leaf lamina with necrotic areas were the symptoms observed on detached leaves treated with the culture filtrate. Only wilting was noticed at higher dilutions. The solvent extractable metabolites produced by *A. alternata* were inhibitory to the test bacteria. The inhibition zones recorded in plates seeded with *B. megaterium* and *B. subtilis* were 0.88 and 1.47 cm<sup>2</sup> respectively.

TLC analysis of the solvent extracts revealed the existence of three phytotoxic compounds: (i) A brown coloured elongated spot with an  $R_f$  value extending from 0.25 to 0.36, (ii) and (iii) Compounds with blue fluorescence having  $R_f$  values of 0.39 and 0.56. Tests with ethanolic ferric chloride, *p*-anisaldehyde and UV spectral characteristics (peaks at 239 and 279 nm in water and 217 and 277 nm in ethanol) confirm the identity of the brown coloured compound as tenuazonic acid. TLC characteristics of the other two compounds did not coincide with those of alternariol and alternariol monomethyl ether. Besides phytotoxicity<sup>11</sup>, the mycotoxic nature of tenuazonic acid is also well established in recent years<sup>12</sup>. Among *Alternaria* toxins, only tenuazonic acid is listed in the Registry of toxic effects of chemical substances<sup>13</sup>. The natural occurrence of tenuazonic acid in blast diseased rice plants<sup>14</sup> and tomato paste<sup>9</sup> was also reported.

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