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Anther development and microsporogenesis in *Swietenia macrophylla* King. (Meliaceae): A histological study**Mahantesh Shitaram Chavhan, Shekappa Ningappa Agadi****Developmental Biology and Histochemistry Laboratory, P. G. Department of Botany, Karnatak University, Dharwad-580003, Karnataka, India***Key words:** Anther development, Anther wall, Microsporogenesis, Pollen grain, Meliaceae**Received:** August 21, 2026 **Accepted:** August 31, 2026 **Published:** September 09, 2026**DOI:** <https://dx.doi.org/10.12692/ijb/29.3.71-86>**ABSTRACT**

The development of anther and microsporogenesis are fundamental biological processes that play a key role in successful male reproduction in flowering plants. *Swietenia macrophylla* King. (Meliaceae) is an economically important endangered species with significant medicinal and commercial value. Despite extensive research on this species, detailed information on male reproductive development of this species is lacking. The present study aimed to investigate anatomical changes across progressive stages of anther development and microsporogenesis in *S. macrophylla* using light microscopy. Anthers of *S. macrophylla* are dithecous and tetrasporangiate. Anther wall formation confirmed basic type, comprising epidermis, endothecium, two middle layers and uniseriate tapetum. Tapetum is secretory type with uninucleate cells. However, it becomes binucleated at tetrad stage and partially bilayered at young pollen grain stage. Microsporogenesis followed the simultaneous cytokinesis producing tetrahedral tetrads enclosed within a callose wall. Tapetum and one middle layer degenerate completely during anther development while epidermis, endothecium and one middle layer persist at anthesis. Mature pollen grains were spheroidal in outline, isopolar and 4(5)-colpate with psilate exine surface. Endothecium developed fibrous thickenings and epidermal cells became papillate at the time of anther dehiscence. Anther dehiscence occurred through the stomium by longitudinal splitting of anther wall.

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INTRODUCTION

The development of functional male gametophyte (pollen grain) is critical for reproduction in flowering plants since pollen plays an important role in double fertilization and seed set (Gomez *et al.*, 2015; Cui *et al.*, 2024). The process of anther morphogenesis and pollen development in angiosperms is highly conserved and involves precisely regulated developmental events (Cui *et al.*, 2024; Astrand *et al.*, 2021).

The development of anther is a dynamic developmental process which involves the establishment of stamen identity within the floral meristem and morphogenesis of anther wherein microspores and pollen grains are produced through meiosis and mitotic cell divisions. In general, ontogeny of anther begins with initiation of the anther primordium from floral meristem which comprises three distinct layers of meristematic cells: the first layer develops into an epidermis, the second layer gives rise to mass of microspore mother cells and three distinct layers of the anther wall namely tapetum, middle layers and endothecium while the third layer differentiates into connective and vascular tissue (Kim *et al.*, 2016; Chang *et al.*, 2016). Therefore, following the establishment of anther morphology, each microsporangium comprises reproductive microspore mother cells which are enclosed within four distinct somatic layers of cells such as tapetum, middle layers, endothecium and epidermis (Yang *et al.*, 2018; Chawla *et al.*, 2017). Ontogeny of an anther is a sequential process that involves coordinated differentiation of sporophytic and gametophytic tissues; both of these tissues function cooperatively to form functional pollen grains (Yang *et al.*, 2018). Mature pollen grains produce and transfer male gametes to the targeted cells of the embryo sac during double fertilization which leads to seed set, thereby influencing plant fertility and agricultural productivity (Borg *et al.*, 2009; Borg and Twell, 2010; Dukowic-Schulze and van der Linde, 2021).

Throughout its ontogeny, anther exhibits remarkable histological diversity and tightly controlled

developmental events at specific stages of development which have received considerable attention for their critical role in the development and distribution of functional pollen grains. Formation of a defective pollen grain can affect the efficiency of successful pollination and fertilization in higher plants. Furthermore, disruption of microspore development or dysfunction of peripheral anther tissues, particularly the tapetum, can lead to male sterility and reproductive failure in several plants (Dogan and Vardar, 2023). Although the developmental pattern of anther and male gametophyte is broadly conserved in angiosperms, some of the embryological characteristics differ from species to species (Astrand *et al.*, 2021). Developmental features such as pattern of anther wall development, type of tapetum, timing of tapetal degeneration, type of cytokinesis during meiosis of microsporocytes, organization of tetrads in callose, condition of pollen grain during anthesis, number of pollen apertures and mechanism of anther dehiscence are considered important embryological features in taxonomic and systematic studies of plants (Çetinbaş and Ünal, 2015). Furthermore, detailed histological analyses during anther ontogenesis gives critical insight into male reproductive biology of Meliaceae species and enhance our understanding of the evolutionary history and relationships within the family. In spite of the importance, comprehensive embryological studies addressing developmental events during anther development and microsporogenesis are limited in Meliaceae. Some of the previously reported embryological studies on species of Meliaceae include Garudamma (1956, 1957), Narayana (1958), Nair (1959), Ghosh (1973), Galati *et al.* (2011), Talawar and Agadi (2018, 2019), and Gama *et al.* (2022).

Swietenia macrophylla King. is an economically important species of the family Meliaceae, renowned for its high-quality timber, distributed in tropical and subtropical regions worldwide (Moghadamtousi *et al.*, 2013). Different parts of this plant possess significant phytomedicinal properties and are used as a therapeutic source against several diseases

(Moghadamtousi *et al.*, 2013). This plant has been assessed as an endangered species under the IUCN Red List of Threatened Species due to overexploitation. In addition, this species has also been classified under Appendix II of CITES (Convention on International Trade in Endangered Species) in 2003 to control unsustainable logging and timber trade of this species worldwide (Madrigal-Villalobos *et al.*, 2025). Although *S. macrophylla* has been investigated from several perspectives, detailed information on male reproductive development in this species is lacking. Therefore, the present study aims to investigate histological events occurring during successive stages of anther development and microsporogenesis in *S. macrophylla*.

MATERIALS AND METHODS

Flower buds of *S. macrophylla* at different developmental stages were collected early in the morning from Botanical Garden of Karnatak University Campus, Dharwad, India, and were immediately fixed in FAA (Formalin-Acetic acid-Alcohol) for 24 h at room temperature. Subsequently, flower buds were preserved in 70% alcohol for longer usage. Later, floral buds were processed for dehydration and infiltration in graded Alcohol : Butanol series using standard plant microtechnique protocol given by Bhandari (1997) and Khasim (2002) and finally embedded in paraffin wax. Transverse sections of flower buds were taken at 6 µm thickness using Leica automatic rotary microtome (RM2145). Paraffin ribbon with anther sections were then affixed to glass slides using gelatin adhesive. Anther sections were stained with safranin and toluidine blue. For toluidine blue, the staining method of Feder and O'Brien (1968) was adopted. Anther sections were deparaffinized in pure xylene followed by rehydration in alcohol grades (100–50%) and finally in distilled water for 3 min in each step. Rehydrated sections were then stained with 0.05% toluidine blue prepared in benzoate buffer (pH 4.4) for 1–5 min. Sections were then washed in tap water for 2–3 min and then rinsed in distilled water for 1 min. Sections were then air dried at room temperature for 10–15 min. Later, air-dried sections

were cleared in pure xylene and mounted with DPX mountant and covered with cover glass. Stained sections were observed under Olympus CX23 light microscope and photographed with the help of Capture 2.4 digital microscope camera.

Safranin staining was performed using staining procedure given by Ma *et al.* (1993) with the slight modifications. Sections were deparaffinized in pure xylene and rehydrated. Rehydrated sections were stained with 1% aqueous safranin for 5–10 min. Subsequently, sections were washed in tap water for 2–3 min and then rinsed in distilled water for one minute. Counterstaining with fast green was omitted, and sections were then air-dried at room temperature for 10–15 min. Later, sections were cleared in pure xylene and mounted with DPX mountant. Periodic Acid-schiff (PAS) staining was also performed for few sections using staining procedure of Feder and O'Brien (1968) for clear visualization of tissues at some stages of anther development. Stained sections were observed under the microscope and photographed.

For scanning electron microscopy (SEM), pollen and anther samples were prepared according to the acetolysis procedure described by Erdtman (1960). Acetolysed pollens and anthers were dehydrated in graded alcohol and then air-dried. Dried pollen grains and anthers were mounted on aluminium stubs and then sputter coated with gold. After sputtering was completed, stubs were taken out of the sputter coater and observed under SEM (JSM-IT500LA) and photomicrographs were captured.

RESULTS

S. macrophylla possesses ten stamens, organized into a cylindrical tube with anthers arranged at the tip alternating with dented projections. Anthers are dithecous and tetrasporangiate (Fig. 1F&G). Process of anther development and microsporogenesis was categorized into ten distinct stages based on developmental characteristics which include primordial stage, archesporial cell stage, early sporogenous stage, late sporogenous stage, microspore mother cell (MMC)

stage, microspore tetrad stage, young microspore stage, vacuolated microspore stage, young pollen grain stage and mature pollen grain stage.

Primordial stage

The young anther primordium which is formed from floral meristem appeared ovoid in outline in

transverse sections. Anther primordium was composed of homogeneous mass of meristematic cells bounded by a distinct epidermis. Cells were mostly polygonal in shape with dense cytoplasm and centrally located conspicuous nuclei, and were compactly arranged without intercellular spaces between them (Fig. 2A).

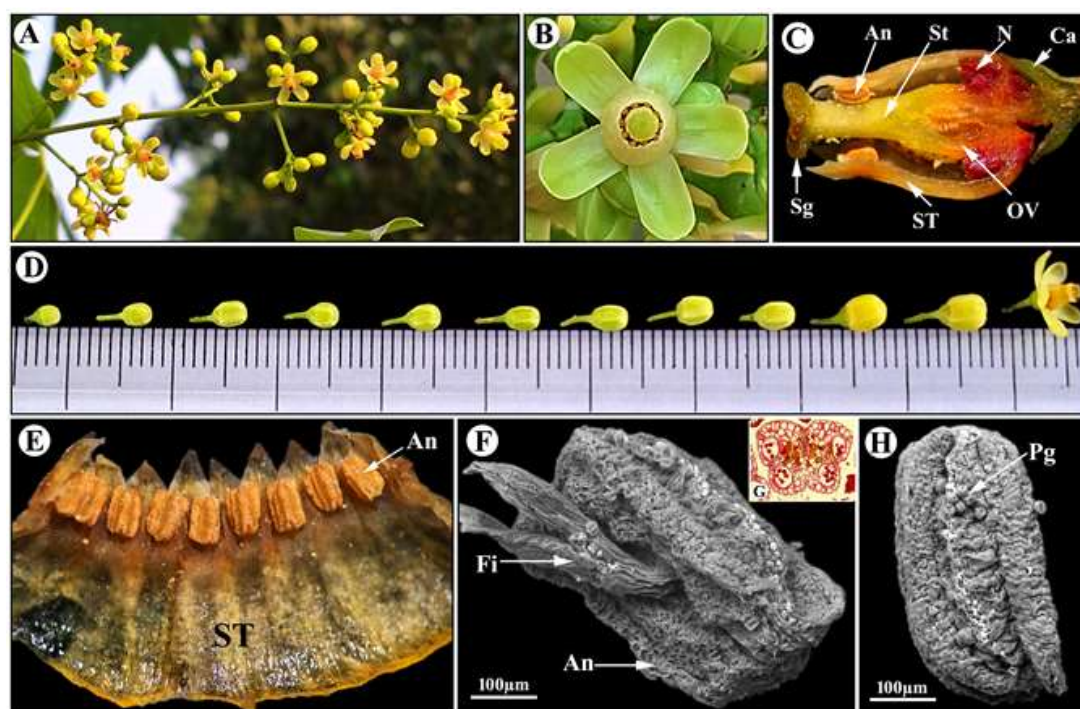


Fig. 1. Floral and reproductive morphology of *Swietenia macrophylla*. (A) Inflorescence. (B) Mature flower in apical view. (C) LS of flower at anthesis showing reproductive structures. (D) Flower buds at different developmental stages. (E) Dissected staminal tube bearing anthers. (F) SEM micrograph of mature anther. (G) Transverse section of mature anther showing four microsporangia. (H) SEM micrograph showing longitudinal dehiscence of anther. Abbreviations: An, Anther; Ca, Calyx; Fi, Filament; N, Nectary; OV, Ovary; Pg, Pollen grain; Sg, Stigma; St, Style; ST, Staminal tube.

Archeporial cell stage

As the development proceeded, ovoid anther primordia became rectangular in shape and cells of the anther primordia were organized into three meristematic layers referred to as L1, L2 and L3 (Fig. 2B). In the subsequent developmental stage, anther assumed slightly four lobed structure. At this stage, one of the hypodermal cells of L2 at each lobe of anther primordium became enlarged and differentiated into an archesporial cell which had a dense cytoplasm with centrally located prominent nucleus. Concurrently, differentiation of connective tissue with vascular cells was noticed in L3 (Fig. 2C). At the following stage, the archesporial cell cut periclinally

(with reference to epidermis) to form the primary parietal cell towards the epidermis and the primary sporogenous cell towards the connective (Fig. 2D). Later, the primary parietal cell divided by periclinal division and formed the outer secondary parietal cell and the inner secondary parietal cell. Concurrently, the primary sporogenous cell also underwent mitotic division (Fig. 2E). As the development advanced, the outer secondary parietal layer again divided periclinally to form the endothecium and one middle layer while the inner secondary parietal layer cut periclinally and gave rise to one more middle layer and the inner most tapetum (Fig. 2F).

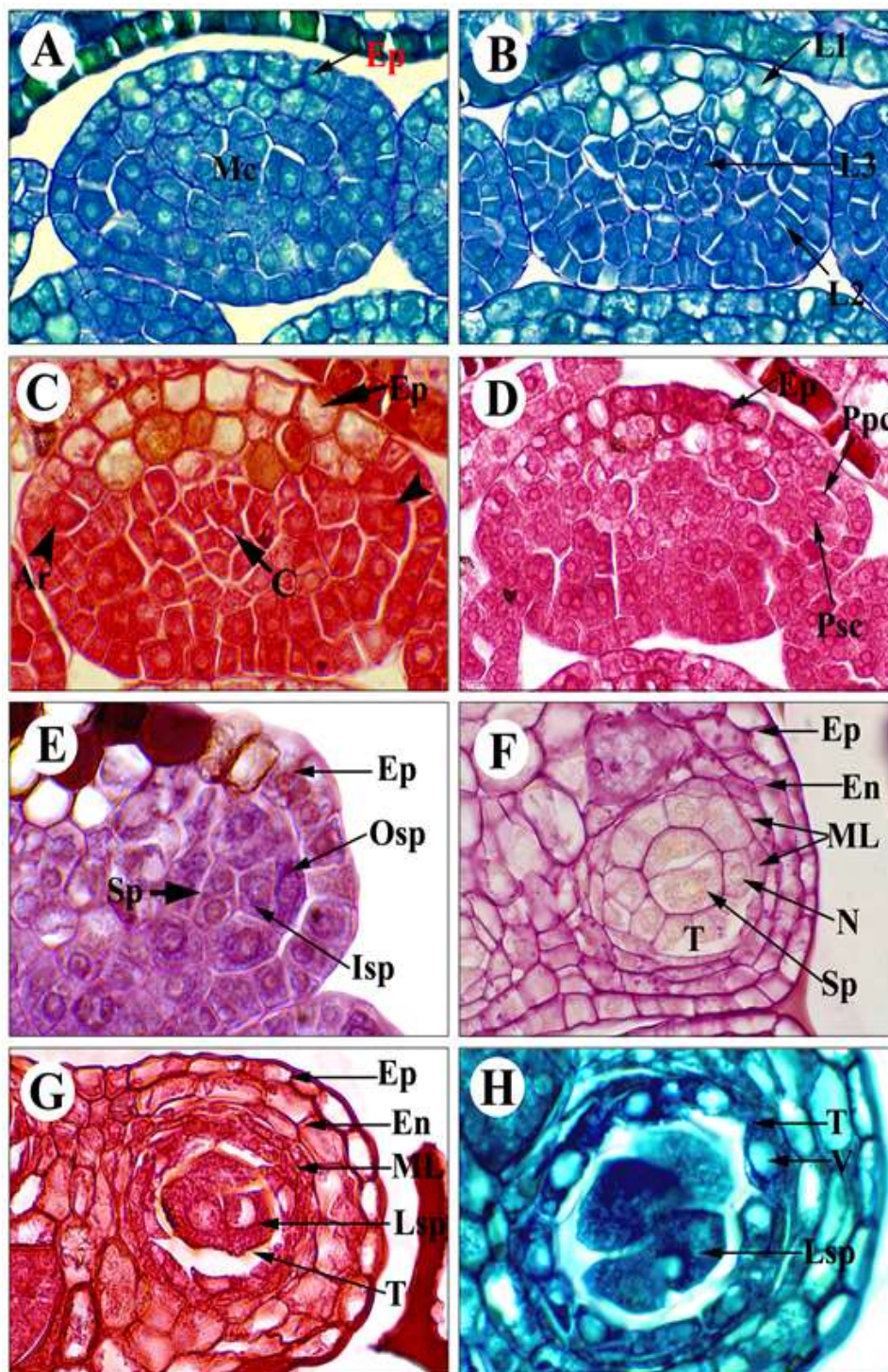


Fig. 2. Transverse sections of *S. macrophylla* anther at successive stages of development stained with safranin, toluidine blue and PAS. (A) Anther primordium with undifferentiated mass of meristematic cells. (B) Rectangular anther primordium with meristematic cells organized into three layers (L1, L2 and L3). (C) Undifferentiated anther with archesporial cells. (D) Undifferentiated anther showing primary parietal cell towards epidermis and primary sporogenous cell towards connective. (E) Anther lobe showing division of sporogenous cells and primary parietal cell into outer secondary parietal layer and inner secondary parietal layer. (F) Anther locule at early sporogenous stage with completely differentiated anther wall layers and sporogenous tissue. Note tapetal cells are uninucleate. (G) Anther at late sporogenous stage. (H) Close-up view of

anther locule at late sporogenous stage showing presence of vacuoles in tapetum. Abbreviations: Ar, Archeporsial cell; C, Connective; Ep, Epidermis; En, Endothecium; Isp, Inner secondary parietal layer; L1, Layer 1; L2, Layer 2; L3, Layer 3; Lsp, Late sporogenous tissue; Mc, Mass of cells; ML, Middle layers; N, Nucleus; Osp, Outer secondary parietal layer; Ppc, Primary parietal cell; Psc, Primary sporogenous cell; Sp, Sporogenous tissue; T, Tapetum; V, Vacuole.

Early sporogenous stage

At this stage, anther was fully differentiated into four microsporangia with well differentiated sporogenous tissue enclosed by four distinct anther wall layers such as epidermis, endothecium, two middle layers and single layered tapetum. Tapetum is secretory type comprising cells with a single nucleus. The sporogenous cells are indefinite in shape, arranged compactly without intercellular spaces, and are directly in contact with tapetum. The sporogenous cells have thin cell walls and are comparatively larger than the cells of anther wall layers. Presence of secretory cells was observed at connective region. These are specialized parenchymatous cells with dense cytoplasm and are relatively larger than connective cells (Fig. 2F).

Late sporogenous stage

At this stage, sporogenous cells before transforming into microspore mother cells increased in size and had dense cytoplasm with centrally located conspicuous nuclei and a large vacuole (Fig. 2G). Tapetal cells, compared to the previous stage slightly enlarged with dense cytoplasm and started vacuolation. Inner middle layer adjacent to the tapetum was compressed and disorganized (Fig. 2H).

Microspore mother cell (MMC) stage

At this stage, sporogenous cells reached maximum growth and transformed into microspore mother cells (MMCs). A characteristic feature observed at this stage was the formation of extracellular callose wall around meiocytes. Deposition of callose wall separated MMCs from each other and also from surrounding tapetum layer. MMCs were irregular in outline, possessed dense cytoplasm with a prominent nucleus and vacuole at the centre. Tapetum layer continued to be vacuolated with dense cytoplasm which was organized towards the periphery of the cell

whereas vacuole occupied almost half of the cellular volume and was organized towards MMCs. One middle layer degenerated completely at this stage while other remained intact in position (Fig. 3A).

Microspore tetrad stage

Microspore mother cells (MMCs) of *S. macrophylla* underwent meiosis with simultaneous cytokinesis which led to tetrahedral microspore tetrads which were encased within a callose wall (Fig. 3D). The first meiotic division in MMCs was not accompanied by cytokinesis; as a result two haploid nuclei were formed within the cytoplasm of individual MMCs (Fig. 3B). At this stage, tapetal cells were intact and remained vacuolated with scanty cytoplasm. Subsequently, these binucleated MMCs underwent meiosis II which was followed by simultaneous cytokinesis (Fig. 3C); as a result four haploid microspores were formed in tetrahedral arrangement. Microspores within tetrads were oval in shape with dense cytoplasm and a centrally located nucleus. Tapetum was structurally intact and appeared metabolically active with increased cytoplasmic density. Vacuoles of tapetal cells were reduced in size and were less abundant (Fig. 3E). Some of the tapetal cells became binucleated due to nuclear division without cytokinesis (Fig. 3F).

Young microspore stage

Following the breakdown of callose wall around tetrads, microspores were liberated into the anther locule. Later, free microspores became spherical in shape and enlarged with dense cytoplasm and a centrally located prominent nucleus (Fig. 3G). Microspore wall at this stage contained both intine and exine layers (Fig. 3H). Tapetum layer remained intact with dense cytoplasm. Vacuoles from tapetal cells disappeared at this stage. Cells of endothecium layer became slightly enlarged; consequently, cells of the second middle layer got compressed.

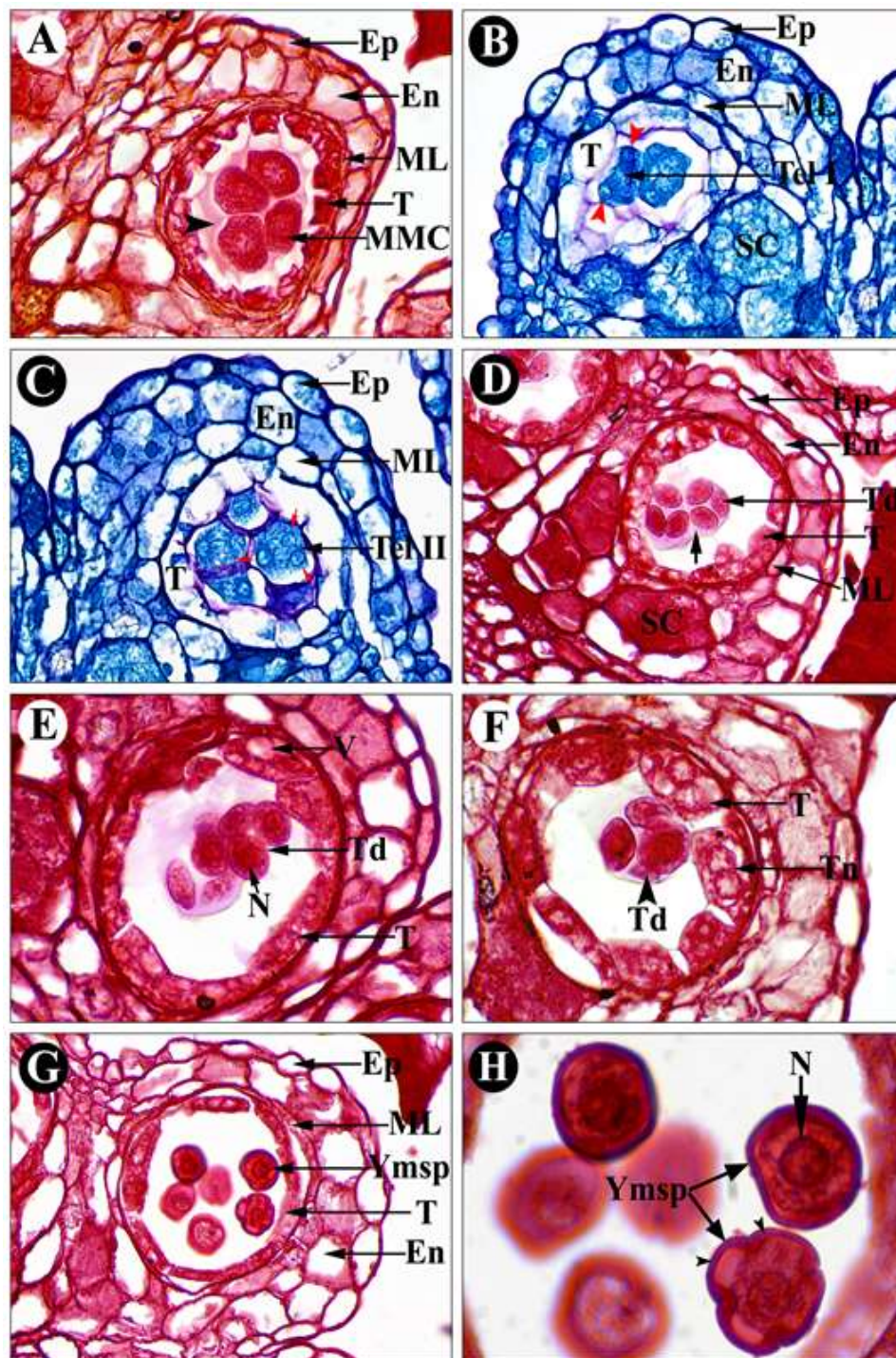


Fig. 3. Transverse sections of *S. macrophylla* anther at successive stages of development stained with safranin, toluidine blue and PAS. (A) Microspore mother cell stage. Note microspore mother cells are surrounded by callose wall (black arrow head), and one middle layer is completely degenerated. (B-C) Microspore mother cells undergoing meiosis: (B) Telophase I (red arrow heads). (C) Cells at Telophase II undergoing simultaneous cytokinesis (red arrow heads). (D-F) Microspore tetrad stage: (D) Tetrahedral tetrads enclosed within callose wall (black arrow). (E) Tapetal cells at tetrad stage showing dense cytoplasm with reduced vacuoles. (F) Tapetum at tetrad stage showing binucleated cells. (G) Young microspore stage. (H) Enlarged view of young microspores with centrally located nucleus. Note cell wall of microspore containing exine and intine layers (black arrow heads). Abbreviations: MMC, Microspore mother cells; SC, Secretory cells; Tel I, Telophase I; Tel II, Telophase II; Td, Tetrads; Tn, Tapetum nuclei; Ymsp, Young microspores.

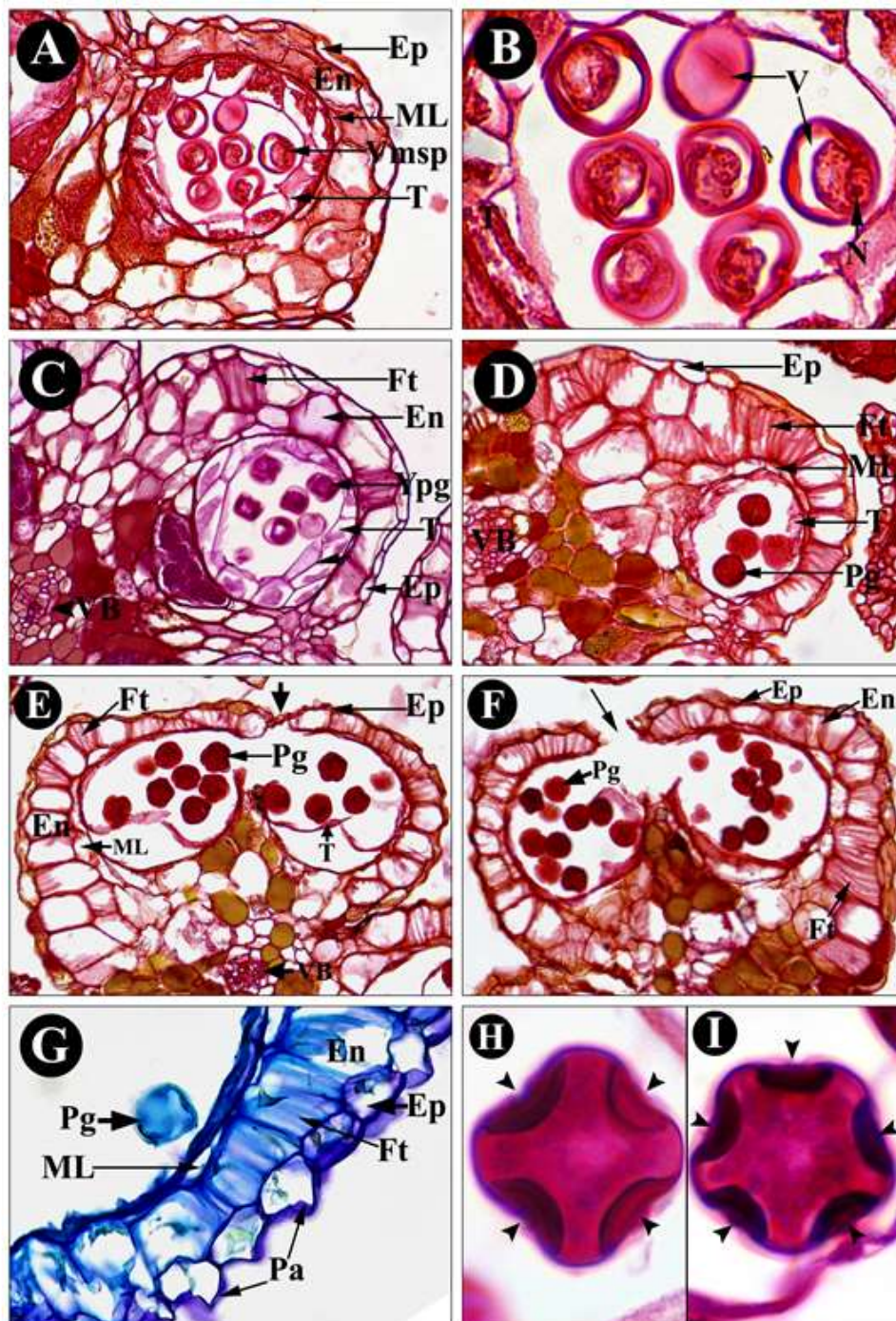


Fig. 4. Transverse sections of *S. macrophylla* anther at successive stages of development stained with safranin, toluidine blue and PAS. (A) Vacuolated microspore stage. (B) Close-up of vacuolated microspores showing large vacuole at the centre pushing nucleus and cytoplasm to the periphery of the cell. (C) Young pollen grain stage. Fibrous thickenings are developing in endothelial cells. Note periclinal division in tapetal cells (black arrow). (D) Anther at matured pollen grain stage showing degeneration of tapetum. (E) Mature anther at anthesis showing formation of the stomium (black arrow). (F) Dehiscent anther showing breakdown of the stomium during dehiscence. (G) Detail of the mature anther wall showing papillate epidermis, fibrous endothecium and persistence of one middle layer. (H-I) Close-up of matured pollen grains showing 4-5 colpi (black arrow heads). Abbreviations: Ft, Fibrous thickenings; Pa, Papillae; Pg, Pollen grains; VB, Vascular bundle; Vmsp, Vacuolated microspores; Ypg, Young pollen grains.

Vacuolated microspore stage

During subsequent development, a large vacuole developed at centre of the microspores. Consequently, cytoplasm and nucleus were displaced to the periphery of the cell (Fig. 4A&B). Microspores were surrounded by distinct intine and exine wall layers. Unlike the young microspore stage, tapetal cells were slightly enlarged with reduced cytoplasm content. Second middle layer got more compressed and appeared here and there.

Young pollen grain stage

With advancing development, vacuolated microspores underwent asymmetric mitotic division and transformed into young (bicellular) pollen grains with vegetative cell and generative cell. Concurrently, large vacuole in the microspore

disappeared gradually and cytoplasm filled the cell. Tapetum layer was still intact in position and became partially bilayered due to periclinal division in some of its constituent cells. Cells of endothecium layer enlarged radially and began to develop fibrous thickenings (Fig. 4C).

Mature pollen grain stage

Mature pollen grains were spheroidal in outline, isopolar with 4(5) elongated colpi surrounded by an intine and psilate exine surface (Fig. 4H-I); Fig. 5A-D). Cytoplasm of the pollen grains was dense and filled with reserved substances. Tapetum layer began to degenerate at this stage (Fig. 4D). The endothelial cells contained profuse fibrous bands of thickenings. Epidermis got compressed and became thin layered due to radial elongation of endothelial cells.

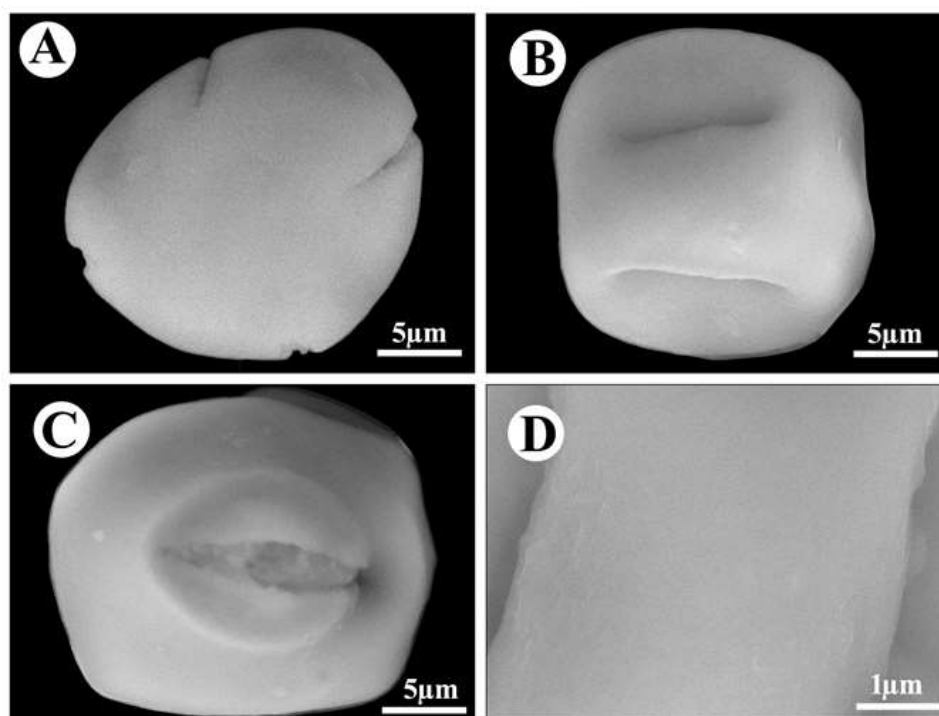


Fig. 5. Scanning electron microscope (SEM) images of pollen grains. (A) Polar view of pollen grain. (B) Equatorial view of pollen grain. (C) Close-up of pollen aperture. (D) Pollen grain with smooth exine surface.

As the anther reached the phase of dehiscence, intersporangial septum degenerated completely and the stomium was formed between two adjacent locules by modification of some epidermal cells. Tapetum layer degenerated completely although thin layer of tapetum remnants remained around pollen

grains (Fig. 4E). One middle layer still persisted along with epidermis and fibrous endothecium. Cells of epidermis layer became papillate during dehiscence (Fig. 4G). Anther dehiscence occurred through the stomium by longitudinal splitting of the anther wall (Fig. 4F).

DISCUSSION

The anther of *S. macrophylla* exhibited general developmental trend in angiosperms. Undifferentiated young anther primordium was organized into three meristematic layers referred to as L1, L2 and L3. Epidermis and stomium cells were derived from L1 layer while L2 layer differentiated into different anther wall layers and sporogenous tissue; and L3 layer formed connective tissue and vasculature. This is consistent with general pattern of anther differentiation in angiosperms (Astrand *et al.*, 2021; Goldberg *et al.*, 1993). Results of this study confirm that the pattern of anther wall formation in *S. macrophylla* is of basic type, and is typical of the family Meliaceae where both the secondary parietal layers contribute in the formation of middle layers of anther wall. This is consistent with the classification of anther wall formation described by Davis (1966). A similar type of anther wall development has also been reported previously in the Meliaceae species such as *Naregamia alata* (Nair, 1959), *Azadirachta indica* (Talawar and Agadi, 2018), *Melia azedarach* (Garudamma, 1957; Galati *et al.*, 2011; Talawar and Agadi, 2019), and *Trichilia claussenii* (Gama *et al.*, 2022). In general, anther wall development follows a consistent pattern within a family. Therefore, the mode of anther wall development represents a characteristic feature of the specific angiosperm family (Astrand *et al.*, 2021; Çetinbaş and Ünal, 2015). However, there are instances where more than one type of anther wall development has been reported. For example, in *Crescentia cujete* (Junior, 2025) and *Sinofranchetia chinensis* (Zhang *et al.*, 2012) both the basic and dicot type of anther wall formations were observed indicating developmental variations among taxa.

In the present study, completely differentiated anther of *S. macrophylla* was tetrasporangiate with the anther wall composed of five anther wall layers namely, epidermis, endothecium, two middle layers and tapetum. However, the number of anther wall layers changed during the development of anther. One middle layer and tapetum underwent degeneration. Consequently, mature anther during

anthesis possessed epidermis, endothecium and one middle layer. A similar feature was observed in *Melia azedarach* (Talawar and Agadi, 2019). Whereas, in *Azadirachta indica* both middle layers and the tapetum degenerated completely and therefore anther wall at maturity had only endothecium and epidermis (Talawar and Agadi, 2018). Contrastingly, in *Ricinus communis* (Wei *et al.*, 2015) and *Rauvolfia serpentina* (Ghimire *et al.*, 2011) anther, the tapetum, middle layer and epidermis degenerated completely and only the endothecium with fibrous thickenings persists at the time of anther dehiscence.

Epidermis of *S. macrophylla* is uniseriate with rectangular cells, remains intact until the end of anther development. During anther maturation, epidermal layer became thinner and papillate. This is probably due to the mechanical tension created by enlargement of endothelial cells and formation of fibrous thickenings in these cells. A similar feature was reported in the anthers of *Melia azedarach* (Galati *et al.*, 2011; Talawar and Agadi, 2019) and *Trichilia claussenii* (Gama *et al.*, 2022). As observed in the present study, the endothecium was single layered, and cells of this layer became enlarged at young pollen grain stage and developed fibrous bands of thickenings. Whereas, in *Melia azedarach* (Talawar and Agadi, 2019) and *Azadirachta indica* (Talawar and Agadi, 2018) formation of endothelial thickenings occurred at mature pollen grain stage. The formation of fibrous thickenings in hypodermal endothecium is crucial for anther dehiscence as these thickenings create mechanical force to break the stomium during anther dehiscence, thereby facilitating pollen dispersion (Dawson *et al.*, 1999; Mitsuda *et al.*, 2005; Yang *et al.*, 2007; Astrand *et al.*, 2021).

Anther of *S. macrophylla* possessed two middle layers. However, at late sporogenous stage, one of the middle layers adjacent to the tapetum was compressed and disorganized due to enlargement of sporogenous cells and tapetum, and finally disappeared during microspore mother cell stage while another one persisted until anther dehiscence.

By contrast, anthers of *Azadirachta indica* (Talawar and Agadi, 2018) and *Melia azedarach* (Talawar and Agadi, 2019) had three middle layers. In the former, all the three middle layers got degenerated during anther maturation whereas in the latter, two middle layers degenerated and one remained until the anther dehiscence.

In the majority of the flowering plant species one type of tapetum is found and is exclusively uniseriate (Astrand *et al.*, 2021). Tapetum in *S. macrophylla* is a secretory type and is single layered which is consistent with the other members of Meliaceae. However, at young pollen grain stage, the tapetum appeared partially bilayered due to periclinal division in some cells of this layer. A similar pattern was also observed in *Melia azedarach* (Talawar and Agadi, 2019), *Rauvolfia serpentina* (Ghimire *et al.*, 2011) and *Passiflora foetida* (Padhye and Deshpande, 1960). However, in *Carthamus tinctorious* (Yeung *et al.*, 2011) two types of tapeta have been reported.

The results showed that, tapetum was uninucleated in pre-meiotic anther but later it became binucleated at tetrad stage due to nuclear division without cytokinesis. A similar observation was made in *Trichilia pallida* (Souza *et al.*, 2001). Similarly, in *Melia azedarach* (Talawar and Agadi, 2019) tapetum was uninucleated but later at vacuolated stage tapetal cells became binucleated. However, Garudamma (1957) reported uninucleate tapetum in *Melia azedarach* but Galati *et al.* (2011) recorded a multinucleate condition with nuclear fusion in tapetal cells of *Melia azedarach*. Likewise, Nair (1959) observed multinucleate tapetum in *Naregamia alata*.

Apart from the species of Meliaceae, similar structural transformation of tapetal cells with varying number of nuclei at different stages of anther development have also been reported in many other angiosperm species including *Stellaria media* (Joshi, 1936), *Passiflora foetida* (Padhye and Deshpande, 1960), *Heuchera micrantha* (Vijayraghavan and Ratnaparkhi, 1979), *Zea mays* (Kelliher and Walbot, 2011) and *Catalpa bignonioides* (Konyar, 2017).

Lattar *et al.* (2012) observed multinucleate condition in the tapetum of *Luehea divaricata*. Vardar *et al.* (2013) reported up to four nuclei in the tapetal cells of anther of *Asphodelus aestivus*. In this case, nuclei of tapetal cells divide repeatedly without cytokinesis as a result, multiple nuclei are formed within the same tapetal cell and unite together to form large nucleus with polyploidy. Likewise, Tutuncu Konyar and Dane (2013) also reported multiple nuclei and nuclear fusion in the tapetum of *Campsis radicans*. An increased nuclear number and polyploidization in the tapetum is mainly due to acytokinetic mitotic division and endoreduplication events. This is probably due to metabolic demand to produce more number of desirable genes responsible for the synthesizing of essential molecules which are necessary for the development and maturation of pollen grains. However, precise role of this phenomenon remains unclear (Weiss and Maluszynska, 2001; Kelliher and Walbot, 2011; Parteka *et al.*, 2022).

In the present study, it was observed that the cells of the tapetum layer became highly vacuolated at late sporogenous stage, and these vacuoles persisted until the formation of tetrads and later decomposed during microspore development. The occurrence of vacuoles in tapetum is an uncommon feature observed in flowering plants. Similar observations were made by Zheng *et al.* (2017) in *Bauhinia blakeana* anther. Polowick and Sawhney (1993) also reported the presence of vacuoles with electron dense deposits in the tapetal cells of tomato anthers.

Our results showed that tapetum was intact until young pollen grain stage, and later at mature pollen grain stage it showed the sign of degeneration, and by the time of anther dehiscence, it was completely degenerated leaving behind a thin layer of cellular debris around matured pollen grains, thereby substantiating its role in nutrition supply during pollen maturation. Similar results were reported in *Melia azedarach* (Talawar and Agadi, 2019). These results support the fact that tapetum is nutritive in function and regulates microsporogenesis and pollen development by secreting essential nutritive

substances into the anther locule and finally it undergoes programmed cell death thereby releasing essential biomolecules for biosynthesis of the pollen wall (Wei and Ma, 2023). Proper development and timely degeneration of tapetum are the essential factors for normal development as well as dispersion of pollen grains. Failure of tapetal degeneration can prevent normal anther dehiscence and may cause male sterility (Kim *et al.*, 2010). In the present study, the presence of secretory cells was observed at the connective region of anther. These are specialized parenchymatous cells with dense cytoplasm, differentiate during early anther differentiation and store reserved food material in them. The occurrence of secretory cells at the connective region of anther is a distinct feature that has been previously reported in species of Meliaceae such as *Azadirachta indica* (Talawar and Agadi, 2018) and *Melia azedarach* (Talawar and Agadi, 2019).

Microspore mother cells were invested with an additional callose wall which remained until completion of meiosis. Formation of extracellular callose (β -1,3-glucan) wall around the microspore mother cells is a hallmark observed during microsporogenesis in flowering plants. Callose wall around microsporocytes is known to play a pivotal role in initiation of meiosis, serve as a selective barrier for biomolecules and a template for biosynthesis of pollen coat (Panchaksharappa *et al.*, 1985; Lu *et al.*, 2014). Review of literature suggests that abnormality in callose metabolism during microsporogenesis can lead to the formation of defective pollen grains and male sterility. MMCs underwent meiosis with the simultaneous cytokinesis which resulted in tetrahedral tetrads of microspores which were enclosed within a callose wall. This is consistent with results of other species of Meliaceae such as *Azadirachta indica* (Talawar and Agadi, 2018), *Melia azedarach* (Talawar and Agadi, 2019; Galati *et al.*, 2011) and *Trichilia clausenii* (Gama *et al.*, 2022).

Mature pollen grains of *S. macrophylla* had a dense cytoplasm filled with reserved substances. Pollen wall contained distinct intine and exine layers. Under scanning electron microscopy, pollen grains were

observed to be spheroidal in outline, isopolar with 4(5)-colpate with psilate exine surface. However, Carreira and Secco (1984) previously described pollen of *S. macrophylla* as 4(3)-colporate with psilate exine ornamentation. In the present study, although pollen grains showed four elongated colpi, the presence of ora (pore) could not be resolved under the present SEM observations. Nevertheless, palynological features observed in the present study are similar to the general palynological characteristics of different genera of the Meliaceae (Large and Mabberley, 1994; Gama *et al.*, 2022; Fernandes *et al.*, 2025).

Like other species of Meliaceae, the anther of *S. macrophylla* dehisced through the stomium by longitudinal splitting of anther wall (Talawar and Agadi, 2018, 2019; Galati *et al.*, 2011; Gama *et al.*, 2022). During anther dehiscence, the intersporangial septum which separates two adjacent anther locules, degenerated completely and a cluster of cells between two locules modified to form stomium, suggesting that dehiscence of anther is an intricate process which involves a series of coordinated developmental and degenerative events in the anther tissues including dissolution of intersporangial septum, development of endothelial thickenings, degeneration of middle layers and tapetum either by mechanical crush or programmed cell death (Wang *et al.*, 2015; Marchant and Walbot, 2022).

CONCLUSION

The present investigation represents first report on developmental anatomy of anther development and microsporogenesis in *S. macrophylla*. Results of our study provides comprehensive account on histological and cytological events during male reproductive development including differentiation of archesporial cell, sequential differentiation of anther wall layers and sporogenous tissue, development and degeneration of tapetum, meiotic behavior of microspore mother cells, production of microspore tetrads and subsequent microspore development, maturation of pollen grains and anther dehiscence. Developmental features recorded in the present study such as basic type of anther wall

formation, secretory tapetum with binucleate condition at tetrad stage, simultaneous microsporogenesis, tetrahedral tetrads, tapetum becoming partially bilayered during young pollen grain stage, persistence of one middle layer until anther dehiscence and pollen morphology represents a combination of developmental traits that contributes to embryological characterization of *S. macrophylla*. The results of this study also show that several aspects of male reproductive development in *S. macrophylla* are consistent with those reported in other flowering plants. At the same time some features of anther development in *S. macrophylla* provide comparative information within the Meliaceae family. This study has enhanced our understanding about male reproductive development in *S. macrophylla*, and also findings of this study may serve as baseline information for future plant breeding research and may contribute to the development of conservation strategies for this endangered species.

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REFERENCES

Åstrand J, Knight C, Robson J, Talle B, Wilson ZA. 2021. Evolution and diversity of the angiosperm anther: Trends in function and development. *Plant Reproduction* **34(4)**, 307–319.
DOI: 10.1007/s00497-021-00416-1

Bhandari NN. 1997. Staining Techniques: A Manual. Ultimate Printers, New Delhi.

Borg M, Brownfield L, Twell D. 2009. Male gametophyte development: A molecular perspective. *Journal of Experimental Botany* **60(5)**, 1465–1478.
DOI: 10.1093/jxb/ern355

Borg M, Twell D. 2010. Life after meiosis: Patterning the angiosperm male gametophyte. *Biochemical Society Transactions* **38(2)**, 577–582.
DOI: 10.1042/BST0380577

Carreira LMM, Secco RDS. 1984. Pollen morphology of plants cultivated in the Goeldi Museum Park—III. Meliaceae. *Bulletin of the Museu Paraense Emilio Goeldi, Botany* **1(1–2)**, 5–22.

Çetinbaş A, Ünal M. 2015. Developmental and cytochemical features of male reproductive organ in *Crataegus tanacetifolia* (Lam.) Pers. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **43(2)**, 507–514. DOI: 10.15835/nbha4329880

Chang Z, Chen Z, Yan W, Xie G, Lu J, Wang N, Lu Q, Yao N, Yang G, Xia J, Tang X. 2016. An ABC transporter, OsABCG26, is required for anther cuticle and pollen exine formation and pollen-pistil interactions in rice. *Plant Science* **253**, 21–30.
DOI: 10.1016/j.plantsci.2016.09.006

Chawla M, Verma V, Kapoor M, Kapoor S. 2017. A novel application of periodic acid–Schiff (PAS) staining and fluorescence imaging for analysing tapetum and microspore development. *Histochemistry and Cell Biology* **147(1)**, 103–110.
DOI: 10.1007/s00418-016-1481-0

Cui DL, Xu CX, Wang P, Gao TY, Wang B, Yu TY. 2024. Male gametogenesis in flowering plants. *Frontiers in Sustainable Food Systems* **7**, 1333544.
DOI: 10.3389/fsufs.2023.1333544

Davis GL. 1966. Systematic embryology of Angiosperms. John Wiley and Sons, New York.

Dawson J, Sozen E, Vizir I, Van Waeyenberge S, Wilson ZA, Mulligan BJ. 1999. Characterization and genetic mapping of a mutation (ms35) which prevents anther dehiscence in *Arabidopsis thaliana* by affecting secondary wall thickening in the endothecium. *The New Phytologist* **144(2)**, 213–222.
DOI: 10.1046/j.1469-8137.1999.00507.x

- Dogan B, Vardar F.** 2023. Anther development and cytochemistry of *Hibiscus syriacus* (Malvaceae). *Phytologia Balcanica* **29(1)**, 53.
- Dukowicz-Schulze S, Van der Linde K.** 2021. Oxygen, secreted proteins and small RNAs: Mobile elements that govern anther development. *Plant Reproduction* **34(1)**, 1–19.
DOI: 10.1007/s00497-020-00401-0
- Erdtman G.** 1960. The acetolysis method. A revised description. *Svensk Botanisk Tidskrift* **54**, 561–564.
- Feder NED, O'Brien TP.** 1968. Plant microtechnique: Some principles and new methods. *American Journal of Botany* **55(1)**, 123–142.
DOI: 10.1002/j.1537-2197.1968.tb06952.x
- Fernandes AD, Lopes ACV, Marques VP, Bellonzi TK, Cerdan IP, Magalhaes R, Gasparino EC.** 2025. Pollen morphology of Meliaceae from Brazilian forest fragments of Cerrado, Brazil. *Palynology* **49(3)**, 2456273.
DOI: 10.1080/01916122.2025.2456273
- Galati BG, Rosenfeldt S, Tourn GM.** 2011. Embryological studies in *Melia azedarach* L. The Journal of the Torrey Botanical Society **138(1)**, 26–33.
DOI: 10.3159/TORREY-D-10-00003.1
- Gama RL, El Ottra JHL, Pirani JR, Demarco D.** 2022. Gynodioecy in *Trichilia* (Meliaceae) and a peculiar case of male sterility due to tapetal necrotic cell death. *Brazilian Journal of Botany* **45(1)**, 449–462.
DOI: 10.1007/s40415-021-00746-4
- Garudamma GK.** 1956. Studies in the Meliaceae I. Development of the embryo in *Azadirachta indica* A. Juss. *Journal of Indian Botanical Society* **35**, 222–226.
- Garudamma GK.** 1957. Studies in the Meliaceae. II. Gametogenesis in *Melia azadirachta* L. *Journal of Indian Botanical Society* **36**, 227–231.
- Ghimire B, Ghimire BK, Heo K.** 2011. Microsporogenesis in *Rauwolfia serpentina* (L.) Benth ex Kurz (Apocynaceae): An evidence for dual cytokinesis in microspore mother cells. *Journal of Medicinal Plants Research* **5(3)**, 432–438.
- Ghosh RB.** 1973. Embryological studies in the family Meliaceae. Fertilisation, endosperm and embryogeny in *Walsura trifoliata* (A. Juss) Harms. CSIC - Estacion Experimental de Aula Dei (EEAD) **12(1/2)**, 1–7.
- Goldberg RB, Beals TP, Sanders PM.** 1993. Anther development: Basic principles and practical applications. *The Plant Cell* **5(10)**, 1217–1229.
- Gomez JF, Talle B, Wilson ZA.** 2015. Anther and pollen development: A conserved developmental pathway. *Journal of Integrative Plant Biology* **57(11)**, 876–891. DOI: 10.1111/jipb.12425
- Joshi PC.** 1936. Contribution to the life-history of *Stellaria media* L. *Proceedings of the Indian Academy of Sciences-Section B* **3(1)**, 8–22.
- Junior NSB.** 2025. Anther development in gourd tree (*Crescentia cujete*, Bignoniaceae): Implications for the evolutionary transition between basic and dicotyledonous types of microsporangium wall formation. *Flora* **330**, 152792.
DOI: 10.1016/j.flora.2025.152792
- Kelliher T, Walbot V.** 2011. Emergence and patterning of the five cell types of the *Zea mays* anther locule. *Developmental Biology* **350(1)**, 32–49.
DOI: 10.1016/j.ydbio.2010.11.005
- Khasim SM.** 2002. Botanical microtechnique: Principles and practice. Capital Publishing Company.
- Kim SG, Lee S, Kim YS, Yun DJ, Woo JC, Park CM.** 2010. Activation tagging of an *Arabidopsis* SHI-RELATED SEQUENCE gene produces abnormal anther dehiscence and floral development. *Plant Molecular Biology* **74(4)**, 337–351.

- Kim YJ, Jang MG, Zhu L, Silva J, Zhu XL, Sukweenadhi J, Kwon WS, Yang DC, Zhang DB.** 2016. Cytological characterization of anther development in *Panax ginseng* Meyer. *Protoplasma* **253**, 1111–1124. DOI: 10.1007/s00709-015-0869-3
- Konyar ST.** 2017. An overview of pollen and anther wall development in *Catalpa bignonioides* Walter (Bignoniaceae). *Trakya University Journal of Natural Sciences* **18(2)**, 123–132. DOI: 10.23902/trkjinat.309718
- Large MF, Mabberley DJ.** 1994. The pollen of *Dysoxylum* (Meliaceae): The demise of *Pseudocarapa*. *Botanical Journal of the Linnean Society* **116(1)**, 1–12. DOI: 10.1111/j.1095-8339.1994.tb00418.x
- Lattar EC, Galati BG, Ferrucci MS.** 2012. Ultrastructural study of pollen and anther development in *Luehea divaricata* (Malvaceae, Grewioideae) and its systematic implications: Role of tapetal transfer cells, orbicules and male germ unit. *Flora* **207(12)**, 888–894. DOI: 10.1016/j.flora.2012.10.005
- Lu P, Chai M, Yang J, Ning G, Wang G, Ma H.** 2014. The *Arabidopsis* CALLOSE DEFECTIVE MICROSPORE1 gene is required for male fertility through regulating callose metabolism during microsporogenesis. *Plant Physiology* **164(4)**, 1893–1904. DOI: 10.1104/pp.113.233387
- Ma Y, Sawhney VK, Steeves TA.** 1993. Staining of paraffin-embedded plant material in safranin and fast green without prior removal of the paraffin. *Canadian Journal of Botany* **71(7)**, 996–999.
- Madrigal-Villalobos J, Valdez-Melara M, García-Díaz E, Zuniga-Umana JM, Gatica-Arias A.** 2025. Review of biotechnological advances in mahogany (*Swietenia macrophylla* King): In vitro culture and genetic transformation. *Plant Cell, Tissue and Organ Culture* **160(3)**, 78. DOI: 10.1007/s11240-025-03003-8
- Marchant DB, Walbot V.** 2022. Anther development—the long road to making pollen. *The Plant Cell* **34(12)**, 4677–4695. DOI: 10.1093/plcell/koac287
- Mitsuda N, Seki M, Shinozaki K, Ohme-Takagi M.** 2005. The NAC transcription factors NST1 and NST2 of *Arabidopsis* regulate secondary wall thickenings and are required for anther dehiscence. *The Plant Cell* **17(11)**, 2993–3006. DOI: 10.1105/tpc.105.036004
- Moghadamtousi SZ, Goh BH, Chan CK, Shabab T, Kadir HA.** 2013. Biological activities and phytochemicals of *Swietenia macrophylla* King. *Molecules* **18(9)**, 10465–10483. DOI: 10.3390/molecules180910465
- Nair NC.** 1959. Studies on Meliaceae II. Floral morphology and embryology of *Melia azedarach* Linn.—A reinvestigation. *Journal of Indian Botanical Society*.
- Narayana LL.** 1958. Floral anatomy and embryology of *Cipadessa baccifera* Miq. *Journal of the Indian Botanical Society* **37**, 147–154.
- Padhye MD, Deshpande BG.** 1960. The male and female gametophytes of *Passiflora foetida* Linn. *Proceedings of the Indian Academy of Sciences* **52(4)**, 124–130.
- Panchaksharappa MG, Rudramuniyappa CK, Hegde RR.** 1985. A review on recent advances in histochemistry of anther development. *Journal of Plant Science Research* **1**, 60–83.
- Parteka LM, Mariath JE, Vanzela AL, Silverio A.** 2022. Nuclear variations and tapetum polyploidy related to pollen grain development in *Passiflora* L. (Passifloraceae). *Cell Biology International* **46(3)**, 462–474. DOI: 10.1002/cbin.11748
- Polowick PL, Sawhney VK.** 1993. Differentiation of the tapetum during microsporogenesis in tomato (*Lycopersicon esculentum* Mill.), with special reference to the tapetal cell wall. *Annals of Botany* **72(6)**, 595–605. DOI: 10.1006/anbo.1993.1150

- Souza LAD, Moscheta IS, Mourao KS, Silverio A.** 2001. Morphology and anatomy of the flowers of *Trichilia catigua* A. Juss., *T. elegans* A. Juss. and *T. pallida* Sw. (Meliaceae). Brazilian Archives of Biology and Technology **44**(4), 383–393. DOI: 10.1590/S1516-89132001000400008
- Talawar PL, Agadi SN.** 2018. Anatomy and distribution of polysaccharide during anther development in *Azadirachta indica* A. Juss. The Journal of Indian Botanical Society **97**(1), 12–20.
- Talawar PL, Agadi SN.** 2019. Ultrastructure and histochemistry of anther development in *Melia azedarach* L. International Journal of Life Sciences and Review **5**(11), 162–171. DOI: 10.13040/IJPSR.0975-8232.IJLSR
- Tutuncu Konyar S, Dane F.** 2013. Anther ontogeny in *Campsis radicans* (L.) Seem. (Bignoniaceae). Plant Systematics and Evolution **299**(3), 567–583. DOI: 10.1007/s00606-012-0743-0
- Vardar F, Ismailoglu I, Unal M.** 2013. Anther development and cytochemistry in *Asphodelus aestivus* (Asphodelaceae). Turkish Journal of Botany **37**(2), 306–315. DOI: 10.3906/bot-1202-20
- Vijayaraghavan MR, Ratnaparkhi S.** 1979. Histological dynamics of anther tapetum in *Heuchera micrantha*. Proceedings of the Indian Academy of Sciences-Section B **88**(4), 309–316. DOI: 10.1007/BF03046195
- Wang H, Mao Y, Yang J, He Y.** 2015. TCP24 modulates secondary cell wall thickening and anther endothecium development. Frontiers in Plant Science **6**, 436. DOI: 10.3389/fpls.2015.00436
- Wei D, Xu H, Li R.** 2015. Dynamics of polysaccharides and neutral lipids during anther development in castor (*Ricinus communis*). Journal of the American Society for Horticultural Science **140**(4), 356–361. DOI: 10.21273/JASHS.140.4.356
- Wei S, Ma L.** 2023. Comprehensive insight into tapetum-mediated pollen development in *Arabidopsis thaliana*. Cells **12**(2), 247. DOI: 10.3390/cells12020247
- Weiss H, Maluszynska J.** 2001. Molecular cytogenetic analysis of polyploidization in the anther tapetum of diploid and autotetraploid *Arabidopsis thaliana* plants. Annals of Botany **87**(6), 729–735. DOI: 10.1006/anbo.2001.1402
- Yang C, Xu Z, Song J, Conner K, Vizcay Barrena G, Wilson ZA.** 2007. *Arabidopsis* MYB26/MALE STERILE35 regulates secondary thickening in the endothecium and is essential for anther dehiscence. The Plant Cell **19**(2), 534–548. DOI: 10.1105/tpc.106.046391
- Yang S, Peng L, Bao H, Tian H.** 2018. Cytological features of developing anthers in Rose Balsam. Journal of the American Society for Horticultural Science **143**(2), 95–100. DOI: 10.21273/JASHS04303-17
- Yeung EC, Oinam GS, Yeung SS, Harry I.** 2011. Anther, pollen and tapetum development in safflower, *Carthamus tinctorius* L. Sexual Plant Reproduction **24**(4), 307–317. DOI: 10.1007/s00497-011-0168-x
- Zhang XH, Ren Y, Tian XH.** 2012. Microsporogenesis and megasporogenesis in *Sinofranchetia* (Lardizabalaceae). Flora **207**(3), 197–202. DOI: 10.1016/j.flora.2012.01.005
- Zheng R, Su S, Xiao H, Tian H.** 2017. Polysaccharides and lipids in the developing anthers of Hong Kong orchid tree (*Bauhinia blakeana*). Trees **31**(4), 1295–1301. DOI: 10.1007/s00468-017-1548-0