International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 9 Issue 1, Jan-Feb 2025 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Decoding Microsporogenesis of Earleaf Nightshade with Histochemistry

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ABSTRACT

This study focuses on the microsporogenesis of the Earleaf nightshade, *Solanum mauritianum*, with particular emphasis on the differentiation of the anther wall and sporogenous cells. The development of the anther wall involves the formation of distinct layers, including the epidermis, endothecium, middle layers, and tapetum. The changes occurring in the sporogenous tissue during gametogenesis can be categorized into early sporogenous, late sporogenous, meiocytes, tetrads, microspores, and pollen grains. In *Solanum mauritianum*, the anther is bilobed and tetrasporangiate, with the anther wall development following the Basic type. The tapetum is of the glandular type. Anther dehiscence is characterized by the formation of pores. Microsporogenesis is analyzed alongside the metabolic activity of cells using histochemical techniques. Specifically, the localization of total proteins in sporogenous and vegetative tissues at various stages of anther development is assessed using amido black staining.

KEYWORDS: *Stellate-tomentoes, abaxial, adaxial, Earleaf nightshade, Solanum mauritianum*

How to cite this paper: Deepa R. Mesta "Decoding Microsporogenesis of Earleaf Nightshade with Histochemistry" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-9 | Issue-1, February 2025, pp.289-293, URL: www.ijtsrd.com/papers/ijtsrd73831.pdf

IJTSRD73831

INTRODUCTION

Solanum mauritianum belongs to the Solanaceae family, a pantropical group comprising about 2,780 species. Within this family, *Solanum* serves as the type genus and is highly diverse. These species are recognized for their ability to produce a wide range of secondary metabolites with significant therapeutic potential (Patela et al., 2010; Jaykumar and Murugan, 2017). *Solanum mauritianum*, commonly known as Earleaf nightshade, is an evergreen woody shrub or small tree that typically grows to a height of 2–4 meters with a trunk diameter of 10–12 cm. All parts of the plant are stellate-tomentose, characterized by sessile to long-stalked hairs. The leaves are large, with a greenish velvety upper surface and a greyish tomentose lower surface. The petiole measures 4–6 cm in length, while the lamina is 10–25 × 5–10 cm, ovate to elliptic, with an entire margin, acuminate apex, and cuneate, often oblique, base. In the leaf axils, petioles of 3–9 cm are accompanied by 1 or 2 smaller, sessile, auriculate leaves, resembling ears, which give the plant its common name.

Numerous bioactive compounds with antifungal, antioxidant, cytotoxic, anti-Alzheimer's, and anti-inflammatory properties have been isolated from *Solanum mauritianum* (Jayakumar and Murugan, 2016, 2017; Ticona et al., 2021).The anther, a vital reproductive structure in plants, plays a key role in enabling desirable fertility modifications through genetic engineering and hybridization. Histochemistry, a powerful analytical technique, facilitates the study of the chemical composition of cells and tissues while preserving their structural integrity (Coleman, 2000). The therapeutic potential of the metabolites in this species, coupled with the significance of the anther in the field of breeding and the application of histochemical techniques, inspired this research.

MATERIALS AND METHODS

The flower buds of different developmental stages collected from surrounding area of Ooty, State Tamilnadu, fixed in Formalin-Acetic acid Alcohol (FAA) for 12 hours. Employing the standard histochemical procedure, fixed floral buds were dehydrated and infiltrated in Alcohol : Xylene series, and embedded in Paraffin wax. 7µm thick transverse sections of flower buds were taken with the help of automatic rotary microtome. Deparaffinised and transfer the slides to alcohol series (100% to 50%) for

3minutes in each. Now slides were incubated in amidoblack reagent for 5 minutes at room temperature. Transfer the slides to 7% acetic acid for two minutes. Rinse the slides in distilled water and cleared in xylem mounted with DPX.

Observations

The development of the anther wall involves the formation of five distinct layers, including the epidermis, endothecium, middle layers, and tapetum. The changes in the sporogenous tissue during gametogenesis can be divided into stages: primordial, early sporogenous, late sporogenous, meiocytes, tetrads, microspores, and pollen grains. In *Solanum mauritianum*, the anther is bilobed and tetrasporangiate, with the anther wall development following the basic type. The tapetum is glandular in nature. Anther dehiscence occurs through the formation of pores. Microsporogenesis is studied alongside the metabolic activity of the cells using histochemical methods, particularly focusing on the localization of protein in the sporogenous and vegetative tissues at different stages of anther development. Throughout these stages, protein localization shifts, with specific cells such as archesporial, primary parietal, and sporogenous cells showing higher concentrations of proteins compared to others.

Primordial stage

During the primordial stage, the anther primordium shows an even distribution of total proteins across its cells (Fig.1). At each corner, specific cells known as archesporial cells differentiate into primary parietal and primary sporogenous cells, both of which exhibit a higher protein concentration compared to surrounding cells.

Early Sporogenous Stage

At this stage, protein localization becomes more concentrated in the primary sporogenous and primary parietal layers. Young anthers show abundant cytoplasmic proteins in the sporogenous cells. In anther, protein-rich content is observed in the early sporogenous cells, the tapetum, and the wall layers, but not in the connective tissue.(Fig.2)

Late Sporogenous Stage

As development progresses, both sporogenous and differentiating tapetal cells accumulate cytoplasmic proteins (fig.3). In this late stage, tapetal cells form vacuoles, and proteins accumulate along their inner surfaces, while sporogenous cells remain rich in total proteins.

Meiocyte Stage

In early meiocytes, cytoplasmic proteins are abundant, and by this time, multiple small vacuoles have formed in the tapetal cells, which are rich in proteins. Throughout meiosis, meiocytes maintain a high concentration of cytoplasmic proteins (Fig.4), but in the late meiocyte stage, there is a noticeable reduction in protein content in the meiocytes, while the protein levels in tapetal cells remain unchanged (Fig.5).

Tetrad Stage

At the end of meiosis, protein content is low, but during the tetrad stage, it increases once again (Fig.6). After meiosis is complete, microspores regain rich protein content similar to the tapetum (Fig7). Once the callose wall around the tetrads breaks down, the four microspores are released and separate within the locule. (Fig.8) Both young microspores and the tapetum stain heavily with amidoblack, whereas the epidermis and endothecium show moderate staining. The connective tissue also exhibits a high protein concentration. As development continues, the microspores and intact tapetum maintain high levels of protein while the middle wall layers show moderate staining.

Vacuolated Microspore Stage

At this stage, the young microspores, which separate from the tetrad, contain high protein levels .(Fig.9) The disintegrating tapetum also shows a high protein content, while the epidermis, endothecium, and middle layers have relatively lower protein levels. The microspores then develop vacuoles and a prominent nucleus.

Pollen Grain Stage

Pollen grains, which develop from mature microspores, are rich in protein and increase in size.(Fig.10) Apart from the dark-stained pollen grains, most other cells in the anther show low staining, indicating the essential role of protein in pollen grain development for male gamete growth.

Stomium Stage

The formation of fibrous thickenings in endothecial cells and the appearance of the stomium on the lateral side signal pollen release through lateral slits, known as poricidal dehiscence.(Fig.11) The stomium shows intense protein staining, which extends into the middle layers as well. Pollen grains are triporate.(Fig.12)

Discussion and result

In the Primordial Stage, proteins are evenly distributed across the anther primordium, with higher protein concentrations observed in differentiating archesporial cells. During the early sporogenous stage, proteins become more concentrated in the sporogenous and moderate in parietal layers. In a study on *Lycopersicon esculentum* by Zhu Yun et al.

(2015), findings on the sporogenous cell stage are comparable to observations in *Solanum mauritianum* .The tapital cells are uninucleated or binucleated in *Solanum mauritianum*. In the Late Sporogenous Stage, proteins accumulate in both sporogenous and tapetal cells, with vacuole formation in the tapetum. The number of nuclei in tapetal cells is one of the most variable characteristics in anthers, showing inconsistency at the tribal level and sometimes even at the generic level. Olmstead et al. (2008) reported that *Withania* (tribe Physaleae) has two nuclei, *Capsicum* (tribe Capsiceae) has three, and *Atropa* (tribe Hyoscyameae) has four nuclei in its tapetal cells, According to Chawan et al.,(1980) abnormalities in pre-meiotic stages show the tapetal cells either degenerate or become abnormally enlarged much prior to the onset of meiosis. In the Meiocyte Stage, proteins are abundant in early meiocytes but decrease in late meiocytes, while protein levels in tapetal cells remain stable.

At the Tetrad Stage, protein levels drop initially but increase again as microspores develop. After meiosis, microspores regain protein-rich content, similar to the tapetum. The Vacuolated Microspore Stage sees microspores with high protein levels and vacuoles, while the disintegrating tapetum retains high protein content. In the Pollen Grain Stage, pollen grains exhibit rich protein content essential for male gamete growth. Finally, in the Stomium Stage, the presence of fibrous thickenings in the endothecium and stomium indicates pollen release through poricidal dehiscence, with intense protein staining observed in these regions

Conclusion

Histochemical analysis revealed distinct protein localization patterns during the stages of anther development in *Solanum mauritianum*. Protein concentrations were highest in sporogenous cells and the tapetum during early developmental stages, with a significant reduction in protein content as meiocytes mature. Protein levels were restored in the microspores and pollen grains, reflecting their importance in male gamete development. Overall, protein accumulation plays a crucial role in the differentiation of anther tissues and the successful formation of functional pollen grains.

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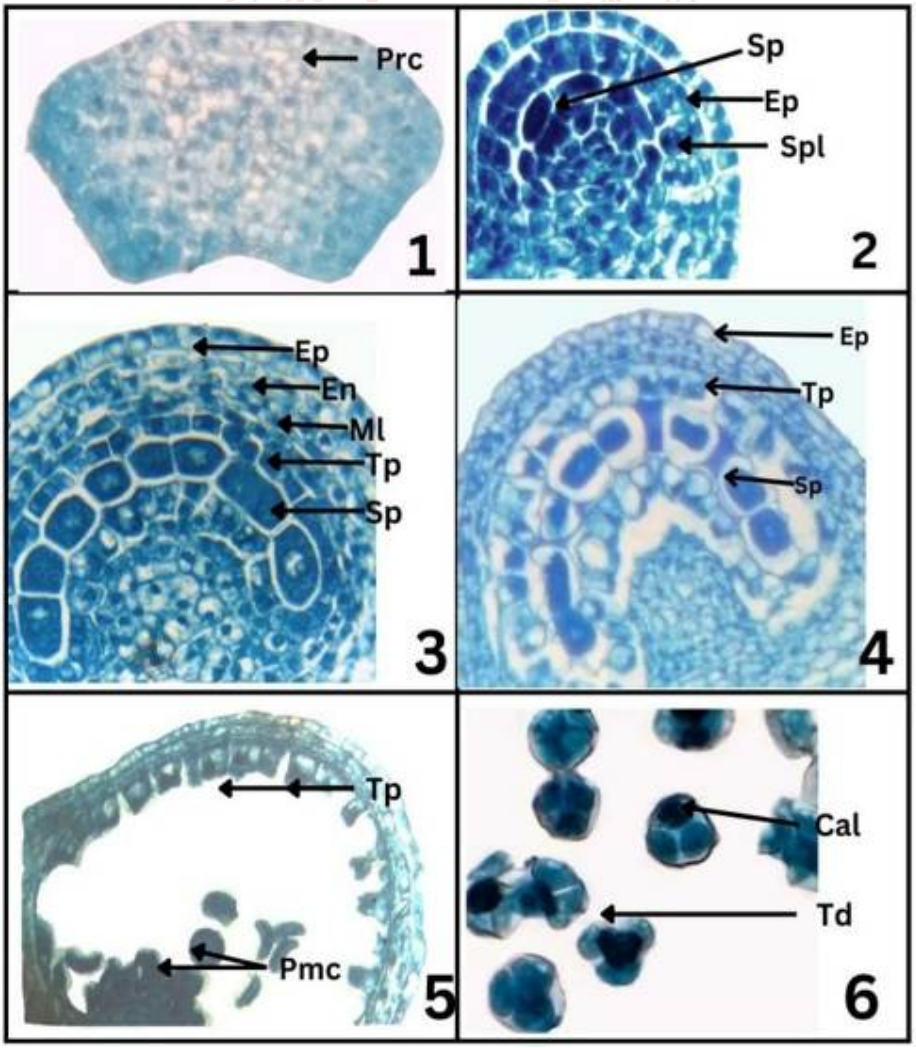
Table1

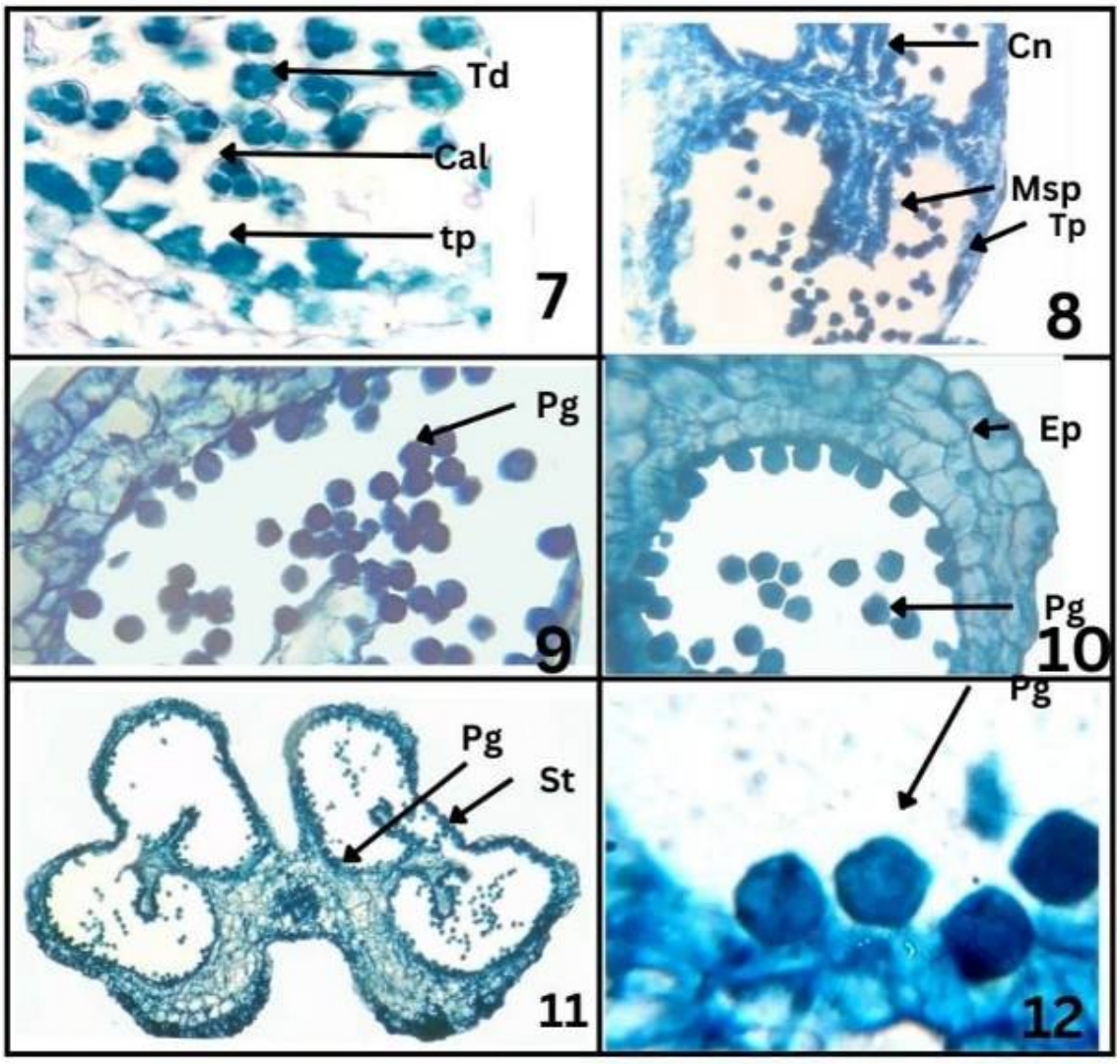


Distributional pattern of total protein in anther development in *Solanum mauritianum* Anther primordium with uniform archesporial cells (Fig.1) Formation of secondary parietal cells (fig.2) Antherwall and sporogeneous

cells(fig..3) Sporogeneous cells get separate in late sporogenous state(fig.4) Microspore mother cell with deforming tapetum(fig.5) Tetrads enrich with total protein(fig.6)

EP=Epidermis, En= Endothecium, Ml= middle layer Sp=Sporogeneouis cells, Tp= Tapetum, Cal=Callose wall, Td= Tetrad, Pmc=Pollen mother cell, Spl=Secondary parital layer

Table2



Distributional pattern of total protein in anther development in *Solanum mauritianum* Microsporangium with tetrads and degenerating tapetum (Fig.7) Microspores (fig.8) Expanding micerospores (fig.9) Pollen grains (fig.10) Tetrasporangiate at dehiscence (fig11) Triporate pollen grain (fig.12)

EP=Epidermis, En= Endothecium, Ml= middle layer Sp=Sporogeneouis cells, Tp= Tapetum, Cal=Callose wall, Td= Tetrad

(10X Fig . 1,2,11 40X Fig 3,4,5,7,8,9,10 100X Fig. 6,12)