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The Histochemical Study of Microsporogenesis in *Solanum seaforthianum* using PAS Method

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Abstract: ***Present study is on the microsporogenesis of Brazilian nightshade Solanum seaforthianum with histochemical analysis. It involves differentiation of anther wall and sporogenous cells. The anther wall development process includes differentiation of wall layers such as the epidermis, endothecium, middle layers, and tapetum. All the change that takes place in the microsporangium during anthesis can be distinguished into three stages 1.Early differentiation, 2. Differentiation during meiosis 3. Post meiotic differentiation. Histochemical staining is widely used to localize proteins, enzymes, polysaccharides, elements and oxidative stress markers. In Solanum Seaforthianum anther is bilobed, tetrasporangiate. The anther wall follows Basic type of development and tapetum is glandular. Microspore mother cells meiotically divided to produce isobilateral and tetrahedral tetrads are found which is rare in dicots. Anther dehiscence is typically porous. Microsporogenesis is studied along with the metabolic potentials of the cells using histochemical methods. Localization of insoluble polysaccharides in sporogenous and vegetative tissues at different stages of anther development achieved by using PAS staining method.***

Keywords: isobilateral, tricolporate, horseshoe shape, papillate

1. Introduction

Solanum seafortianum is a widespread invasive weed belongs to nightshade family Solanaceae consists of about 100 genera together with 2500 representative species (Olmstead & Bohs, 2007). Solanum is hyper genus having economically important species like *Solanum melongena, Solanum tuberosum , Solanum lycopersicum and Capsicum annuum.* Half of the world's populations obtain routine food from these species. Some of species like *Atropa belladonna, Hyoscyamus niger* and *Datura stramonium* are basic component of pharmaceutical world. *Nicotiana tabacum* and *Nicotina rustica* are of economic importance species. Still many solanum species need to be valued for their medicinal value and are under research. Non spiny Brazilian nightshade *Solanum seaforthianum* is one of them.

In the research work of structure and histochemistry of medicinal species of Solanum by Jagatheeswari (2014) conducted a cytological investigation of *Solanum seaforthianum* from Brazil, providing insights into its chromosomal characteristics. Cytological investigation of Brazilian nightshade revealed that all the four types of chromosomes are present in this species. Meiotic behavior of a new tetraploid cytotype of Brazilian nightshade explained by Raman Preet & Raghbir Chand Gupta (2018). P Sivakami Sundari and Sonal Dubey (2022) explored fruits of *Solanum seaforthianum* and *Solanum erianthum* for their phytochemical constituents and investigated for anthelmintic effect. Phytochemical constituents were analyzed using preliminary tests and FTIR. Current research aims to synthesize histochemistry of anther and microsporogenesis of *Solanum seaforthianum* of Solanaceae family. Anther play crucial role in the reproduction of flowering plants. To give clear picture of metabolic potential of cells during anther development histochemical study is essential. It will provide information about intracellular components and answers question about specific role of each tissue. Histochemistry is a methodological approach that allows the chemical analysis of cells and tissues in relation to their structural organization (Coleman 2000). The pattern of localization of histochemical substance is not uniform throughout the course of anther development. Ontogenetic study of anther provides detail information of differentiation of cells and tissues where as histochemical analysis gives localization of promoters in specific tissues. Localization of insoluble polysaccharides in anther at different stages of microsporogenesis achieved by using PAS staining method.

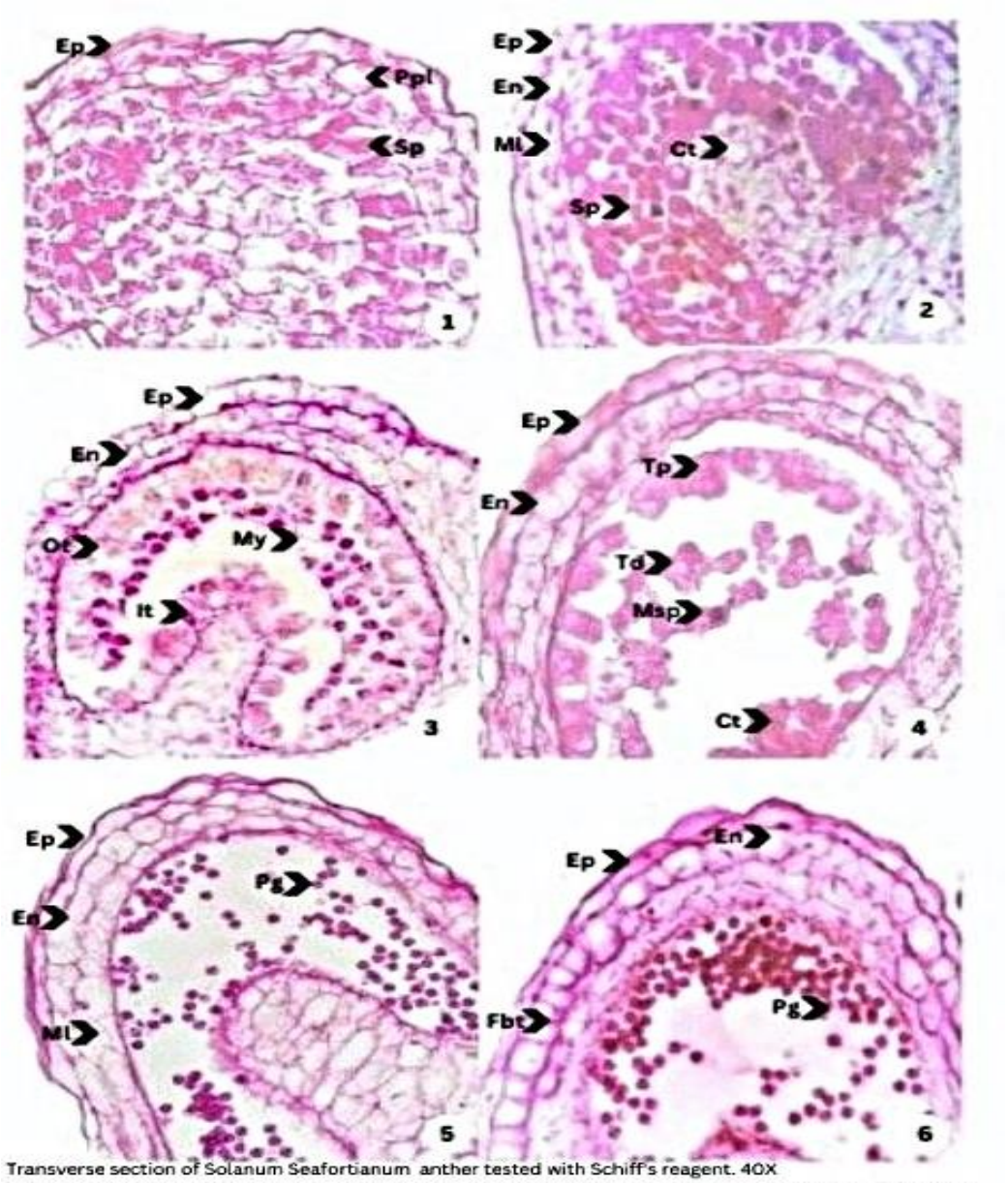
2. Materials and Methods

The flower buds of different developmental stages collected from surrounding area of Hangal, 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. Sections were deparaffinised in Xylene for 30 minutes and then alcohol: xylene series. Then transfer the slides to alcohol series (100% to 50%) for 3minutes in each. Transfer the slides to 0.5% periodic acid for 15minutes then incubated in Schiff's reagent for 15-30 minutes at room temperature. Rinse the slides in distilled water and passing these slides through alchohol series (50% to 100%) procedure reversed. Presence of Carbohydrate in anther walls and sporogenous cells at different stage of anther development confirmed by magenta red color.

3. Observations

The anther development from sporogenous tissue to mature pollen grain in *Solanum seaforthianum* studied under three stages in this. Anther wall differentiated into five prominent layers outer epidermis, hypodermal endothecium, two middle layers and tapetum enclosing four microsporangiate. Sporogenous cells were derived from primary parietal cells. These cells undergo meiosis and form tetrahedral and

isobilateral tetrad released as haploid microspore. During gametogenesis male gametes get matured into pollen grains formed and dehisced. This dithecous anther has four microsporangia and is of horseshoe shaped, due to presence of connective tissues. During the anthers development unique predetermined changes takes place which can be distinguished into three important stages. These changes were divided into 15 stages (Scott et al. 2004) and 18 stages (Laza et al.2022).



Transverse section of Solanum Seafortianum anther tested with Schiff's reagent. 40X
(Ep=Epidermis ; En=Endothecium ; Ml=Middle Layer ; Tp=Tapetum ; Ot=Outer Tapetum; It=Inner Tapetum; My=Meiocyte; Msp=Microspore ; Ct=Connective tissue; Td=Tetrad; Pg=Pollen Grain ; Fbt= Fibrous thickening; Sp= Sporogenous tissue)

1) Early differentiation

Homogenous mass of anther primordium is free from starch undergoes mitotic division forming outer wall layers and inner sporogenous tissue. Cell walls of sporogenous tissue of anther stain intensely for insoluble polysaccharides (Fig. 1) Primary parietal layer originates for successive divisions of archesporial cells leading to form five well-differentiated layers of anther wall. Primary parietal cells have starch grains. Outer epidermis is single layered made up of isodiametric cells. But cells of middle layers are bigger two intermediate layers next to endothecium. The external tapetum/parietal tapetum with cells facing the sporogenous tissue. The internal tapetum/connective tapetum abutting the connective.(Fig;3) It is very different from the parietal tapetum because it presents longer cells. Sporogenous tissue is rich in insoluble polysaccharides. Tapetal cells are having high concentration of insoluble polysaccharides shows that they play important role in nutrition.

2) Differentiation during the meiosis

At the time of intiation of meiosis the meiocytes still possess cytoplasmic polysaccharides. Callose wall deposits around the meiocytes in late sporogenous stage.(Fig. 2) During meiosis tapetal cells undergo reorganization. At the completion of meiosis starch grains disappear in the flattened tapetal cells. The large single vacuole is fragmented into three, four small vacuoles. Starch storage persists in the wall layers and connective. Microspores in tetrads are surrounded by thin callose wall and show distinct PAS positive. Callose wall of tetrad serves to protect the synthesis and then

dissolves by callase. Subsequently, as they separate from tetrad, connective and wall layers namely epidermis, endothecium and middle layers are engorged with polysaccharide grains. Haploid staminoids are poorly stained in early stage and show low metabolism.

3) Post-meiotic differentiation

Microspore tetrad stage terminates when the callose deposition is degenerated. Microspores get free after dissolution of callosic wall. Now middle layer disappears completely. At late vacuolated stage, microspores, including their wall possess rich polysaccharides. At this stage, weakly stained ubisch bodies appear on the surface of degenerating tapetal cells. The cells of middle layer and endothecium enlarge radially and this process is associated with depletion in their starch storage. The reserve substances in connective and wall layers used for endothecial wall thickening, leads to formation of fibrous thickening and exine formation (Fig. 6). Enlarged tricolporate pollen grains released through apical pore (Fig 5). Thus, anther dehiscence is porocidal.

4. Result and Discussion

Histochemistry allows an identification and localization of metabolic contents at cellular level based on the use of specific staining reactions and imaging (Yadav et.al, 2024).This histochemical analysis gives distinctive cellular characteristics associated with anther development. Microsporogenesis of anther is associated with synthesis and digestion of varied types of substance (Vijaylakshmi Naik 2002). Davis (1996) reported that anther is tetra sporangiate in most of angiosperms. It is true in Solanum species of family solanaceae. Anther wall formation is dicotyledonous type or basic type in solanaceae family (Garcia 2002).In *Solanum seafortianum*, primary parietal layer gives rise to middle layer and it follows basic type of anther development. Lilian and Passarelli (2006) revealed that *Solanum glaucophyllum* has papillate epidermis. Anther wall of locule had five layers comprising epidermis, endothecium, two middle layers and tapetum. Similar to *Glaucophyllum*, *Solanum seafortianum* had papillate epidermis. Occurrence of insoluble polysaccharide in sporogenous cells is more than in anther wall layers in initial stages.

Tapetum is of two types, amoeboid and glandular. In the genus Datura, both the types of tapetum were reported. *Solanum Seafortianum* has glandular type of tapetum. At premiotic stages the tapetal cells are binucleated in many species but uninucleated in this species. Tapetum plays important role in productivity and fertility of plant as it provides nutrition to microspore. MALE STERILITY 1(Ms1) is Plant Homeodomain (PHD) finger transcription factor that is crucial to pollen wall formation and tapetum PCD (Yang et al. 2007; Gomez et al. 2015) In the ms1 mutant, degeneration of immature pollen occurs soon after microspore release; the tapetum becomes abnormally vacuolated with altered degeneration and the microspore appear sticky with minimal exine formation suggesting unusual pollen wall composition. (Arizumi and Toriyama (2011); Gomez et al. (2015).

Callose wall appears surrounding the meiocytes in late sporogenous stage and these undergo meiosis. In the present study, at the completion of meiosis the polysaccharides content increases in microspore tetrad and microspores are set free by dissolution of callose wall around the tetrad. At the maturation of pollen grain endothecium develops fibrous thickening. Multi layered endothecium had been reported in taxa *Nicotina glutinosa and Nicotina tobaccum*. No rupture of stomium, anther dehiscence is typically porocidal releasing tricolporate pollen.

5. Conclusion

Among the non-spiny nightshades Brazilian nightshades are pantrophic and having pharmaceutical value. Presence of isobilateral tetrad, dehiscence by apical pore, and occurrence of fibrous thickening in endothecium are special features of the species *Solanum seaforthianum* investigated in this study. Higher concentration of insoluble polysaccharides in tapetal cell confirms its nutritive role. This study enhances the understanding of the fundamental embryology of *Solanum.*

6. Future Scope

This study provides a clear picture of the ontogeny and localization of insoluble polysaccharides during microsporogenesis. It highlights the usefulness of histochemical data for taxonomists in identifying and classifying species within the genus *Solanum*. Specific localization patterns of carbohydrates, lipids, proteins, and phenolic compounds can aid in distinguishing different *Solanum* species, enhancing taxonomic studies. Additionally, variations in the anther may provide insight into phylogenetic relationships.

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