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Ontogeny of anther in *Solanum viarum*, Dunal

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Abstract
During last three decades considerable histological information on anther development were done by many scientists, but histological information on the anther development of Solanaceae members is almost lacking. An attempt is made to understand the development of anther in *Solanum viarum*, or is there any peculiar feature(s) associated with microsporogenesis. In the present study anther wall development is basic type, epidermis and endothecium is single layered and persistent. Middle layers are two in number, tapetum is binucleate glandular, (in the early stage sporogenous bilayered tapetum is seen) dimorphic in nature, dual in origin.
Rich content of carbohydrate is present in archesporial, sporogenous, meiocyte, tapetal cells, wall layers and pollen grains. At sporogenous stage rich content of starch granules in the wall layers, partition wall and connective is observed. It can be concluded that there is mutual interaction and utilization of biochemical substances in the anthers during formation and differentiation of pollen grains.

Keywords: Sporogenous tissue, Endothecium, Meiocyte, Microspore, Pollen grain.

1. Introduction
The study of developmental stages of any plant structure is a fascinating subject because development is a sum of all these events that take place during the life of plant. Anther is an important structure because of the central role played by it in the plant reproduction. The anther produces the male gamete during sexual reproduction.
To know about the relationship between anther structure and pollen development, it is necessary to conduct several studies in cytochemical, histochemical and physiological shields on anthers of different plants (Hansson and El-Ghazaly, 2000, El-Ghazaly, *et al.* 2001) [24, 19].
Study of anther development in angiosperms. Much of the early work is compiled by Maheshwari (1950) [39]; the later studies have been surveyed by Bhandari (1984) [12], Raghavan, (1997) [49].
In general the male gametophyte completes its early development within the anther. The sequential stage of pollen development, microsporocyte and pollen mother cells are produced in the sporogenous tissue within the anther. The two divisions of meiosis transforms these cells in to haploid microspores, each pollen mother cell producing a first dyad and tetrad in the meiotic divisions and result in formation of microspores. The unequal division of microspores takes place (microspores mitosis) forming vegetative cell and generative cell, both of them are included within the cell wall of the original microspore and both are interconnected with micro tubular connections, the generative cell under goes mitotic division within the pollen grain as a result two male sperm cells (Joseph P.1968, Mascarenhas.1989) [33, 41] are formed which are differ in cellular contents that can be termed as cytoplasmic heterospory.
The developmental events of microsporogenesis and pollen formation are exquisitely timed and choreographed, recurring in precise chronological order. That correlates with the floral size (Koltunow *et al.,* 1990, Scott, *et al.,* 1991) [37, 54]. Efforts have frequently been made to describe microsporogenesis and pollen formation. From the cytological, molecular and genetic perspective largely in model plant species.
Davis (1966) [17] observed four different types of anther wall development in angiosperms. Basic type (type I), dicotyledonous (type II), Monocotyledonous (type III), and Reduced type (type IV). In general specific type of anther wall development found in each family however, some families possesses two types of anther development, such the Commelinaceae having type I and type III (Hardy, *et al.,* 2000) [25]. And the family Solanaceae having type I type II (Garcia CG 2002) [22].

The presence of a callose wall around meiocyte is widely regarded as a prerequisite for meiosis in flowering plants. The wall isolates meiocytes from other sporophytic tissues and concurrently, prevents them from dehydration in water stress conditions (Li, *et.al.* 2010) [38]. The callose barrier may serve as a molecular filter that transmits only signals that are indispensable for meiosis into the meiocytes (Dong, *et al.* 2005; Rodriguez-Garcia and Modjeska- Sawka, 2011) [18, 50]. Following degradation of the callosic tetrad walls, the microspores are released into mature pollen grains (Wan, *et al.* 2011; Xie, *et al.*2010) [64, 66]. In the anther locule, free microspores become bicellular pollen grains after asymmetric mitosis and once they have reached maturity, are released by anther dehiscence.

However, Nanetti (1912) [42] in *Solanum muricatum* and Young (1922) [67] in *S. tuberosum* invoked the Lilium type, and this was criticized later by Bhaduri (1932) [11], who reported Polygonum-type development in *S.melongena*. Cooper (1931) [16], Bhaduri (1932, 1935) [11, 10], Satina (1945) [52], Goodspeed (1947) [23], Parashar and Singh (1986) [47], Villari and Messina (1996) [63], Karihaloo and Malik (1996) [34], Garcia CG (2002) [22] and others made significant contributions to the embryology of a variety of Solanaceae species.

To understand the biochemical language this acts as a stimulus for establishment of organogenesis from simple to complex. Some chemical substance which have contributory role in growth and development of S*olanum viarum* characters.

During last three decades considerable histological information on anther development were done by many scientists, but histochemical information on the anther development of Solanaceae members is almost lacking. An attempt is made to understand the development of anther in *Solanum viarum*, or is there any peculiar feature(s) associated with microsporogenesis.

2. Objectives

The present study on development of microsporogenesis and male gametophyte of *Solanum viarum is* taken with following objectives.

1. To characterize the peculiarities of the reproductive structure.
2. To determine whether the development of male gametophyte in *Solanum viarum* is same or deviate from those of other angiosperm species.

3. Materials and Methods

The plant *Solanum viarum* which belongs to Solanaceae family. The flower buds of different developmental stages were collected from Botanical garden, Karnataka University, Dharwad and fixed in the (FAA) for 12 hours. The fixed floral buds were dehydrated and infiltrated in ethanol-butanol series, embedded in paraffin wax. 6µm thick transverse sections of flower buds were taken with the help of automatic rotary microtome. In the present investigation following method is employed to stain.

3.1 PAS method (Feder and O' Brien, 1968; Khasim 2002) [20, 36].

3.1.1 Staining procedure

1. Section were deparaffinized in xylene and hydrated, sections were incubated in 0.5% Periodic acid for 15 minutes at room temperature.
2. Sections were washed in running water and incubated in Schiff's reagent for 15-30Minutes at room temperature.
3. Rinse the slides in distilled water & treated with bleach to remove superfluous stain.
4. Sections were then washed in distilled water, dehydrated with alcohol xylene series, cleared in xylene and mounted with DPX.

3.1.2. Colour indication

Sections stain magenta red.

3.1.2.1 Photomicrography

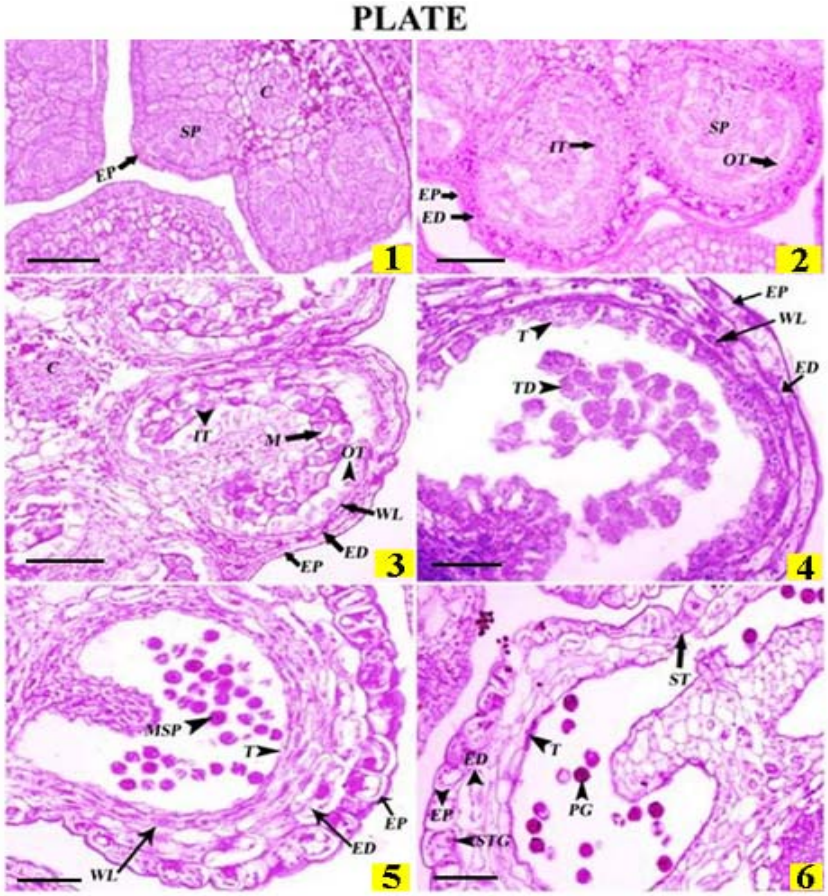
The stained sections were microphotographed using ProGress C5 ZEISS Imager M2 Bright field / Fluorescent Modular microscope.

4. Observations

The anther of *Solanum viarum* is tetrasporangiate. In transverse section of the anther, each anther primordium arises as a small group of undifferentiated cells. Which is oval in shape with a prominent nuclei surrounded by epidermis. Later the oval shaped primordium acquires a four lobed structure, due to differentiation of hypodermal cells in to archesporial cells in the corners of anther primordium, presence of starch grains only at connective (Fig.1).

The development of the anther wall is initiated by the differentiation of the archesporial cells in to inner sporogenous and outer parietal cells. Further the outer primary parietal cells undergoes two or more periclinal divisions and forms secondary outer parietal layer and secondary inner parietal layer. Where secondary outer parietal cell will give rise to outer endothecium and inner middle wall layer and secondary inner parietal cell will give rise to one middle layer and inner most tapetal layer which surrounds the sporogenous tissue. Hence the basic type of wall formation can be seen in *S. viarum.* (Fig.2). The sporogenous tissue produced by the mitotic division of primary sporogenous cells are surrounded by 5 anther wall layers. These cells are seen in the anther as solid mass filling the locule. The epidermis, and endothecium is single layered and persistent throughout the pollen grain formation (Fig.2). There are two ephemeral middle layers and the tapetum is glandular and the cells are binucleate, dimorphic and dual in origin. The meiocyte at the time of meiosis encased in an impermeable Callose wall (β-1-3 Glucan) (Fig.3) and surrounded by tapetum.

Here in this, the first meiotic division is not followed by wall formation. Consequently, a binucleate cell is formed after meiosis –I; there is no dyad formation. The two haploid nuclei synchronously undergo the II - meiotic division and form the tetrads. Meiosis in microspore mother cell results into tetrads of four haploid microspores is still encased within a callose wall (Fig.4). The tetrads are tetrahedral in nature and middle layer has started degenerating in this stage. After dissolution of callose wall, young microspores separate and grow rapidly thus exine wall is synthesized (Fig.5). At the microspore stage enlargement of epidermal and endothelial cell is observed. After maturity the microspores become vacuolated and pollen grains are formed with a definite exine and intine wall and at this time the tapetum degenerates. In Solanaceae members the anther dehiscence is in the form of poricidal nature because of absence of fibrous bands of thickening in the anther wall layers of *Solanum viarum* (Fig.6). Mature pollen grains are two celled at the shedding time.



Plate

Transverse sections of *Solanum viarum* anther tested with Schiff's reagent
(EP = Epidermis; ED = Endothecium; WL = Wall layers; T = Tapetum; IT = Inner tapetum; OT = Outer tapetum; SP = Sporogenous; M = Meiocyte; TD = Tetrad; MSP = Microspores; PG = Pollen grains; ST = Stomium; STG = Starch grains; C = Connective)Bar: Fig. 1 - 6 = 50µm

1. Anther primordium showing starch grains around the connective.
2. Epidermis, endothecium, wall layers, partition walls and connective showing starch storage at early sporogenous stage.
3. Anther showing meiocyte, both inner and outer tapetum shows less starch storage but persisted in epidermis, endothecium, wall layers and connective.
4. Tetrahedral tetrads with the wall layers. Starch grains deposition is seen in epidermis, wall layers, tapetum and connective.
5. Anther showing microspore stage, with the wall layer, degenerating tapetum, enlargement of epidermal and endothelial cells.
6. Pollen grains stage, Epidermis only shows starch grains. Arrow shows stomium.

5. Discussion

The anther is a morphologically simple organ of the flower concerned with microsporogenesis and production of pollen grains, which undergoes a series of morphological and physiological changes until it reaches maturity. During last two to three decades new techniques e.g. molecular biology, genetics etc. have been integrated in the study of microsporogenesis. Among these techniques histochemistry has a special place in the revolutionization of our knowledge about structural and functional aspects of anther. Although considerable histochemical information is available on various aspects of anther development.

The Sequential developmental events of the anther correspond to the underlying sequential biochemical/physiological events. During the growth of the anther, peaks of activity of respiration, macro-molecules (RNA, Proteins and Starch), soluble metabolites and wide range of enzymes occur at predictable and different growth phases. During last 5 decades considerable histochemical information is available on various aspects of anther development (Heslop-Harrison, 1972; Mascarenhas, 1975; Bhandari and Sharma, 1983; Blackman and Yeung, 1983; Panchaksharappa, *et al.,* 1985; Shivanna and Johri, 1985; Hegde, *et al.,* 1993) [30, 41, 13, 14, 46, 57, 26]. Katti, *et al,* 1994; Hegde and Isaacs, 1992; Agadi SN, 1996; Jayaraj, M. 2003, Agadi SN.2012, Agadi and Viayalxmi 2012a, 2012b) [35, 27, 4, 31, 3, 2, 1].

As in majority of Solanaceae the anther is tetrasporangiate. The development of microsporangium wall conforms to the basic type (Davis, 1966) [17]. *Datura* is exceptional only one species was included in basic type, *Datura ceratocaula*, (Garcia CG 1998) [21]. In contrast to Davis' suggestion (1966) [17], the dicotyledonous type is less frequent than the basic type anther wall formation indicating that, the majority of the species with basic type of wall formation characterized the family. The tapetum undergoes nuclear division as the nutritional requirements are increased usually during the meiosis. Meiosis in microspore mother cells accompanied by simultaneous cytokinesis and resulting microspore tetrads are predominantly tetrahedral. In the present study one middle wall layer degenerates and one remains as it is. Wall

formation usually receives little attention in the study of anther ontogeny, with regard to *Solanum*; wall formation has been studied in few genera. The basic type of anther wall development has been observed in *Atropa belladonna*, (Prakash, 1987; Sharma, *et al., 1987*) [48, 56] W*ithania somnifera* (Davis, 1966) [17] *Solanum glaucophyllum* (Garcia CG 2002) [22].

The present ontogenetically study compared with those of other Solanaceae plants, both the dicotyledonous type and basic type of anther wall formation were observed in the members of Solanaceae i.e. *Datura stramonium* and *Datura metal* (Garcia CG 1998 and Thiagarajan, 1986) [21, 58], respectively. But the basic type of anther wall formation is considered as an exception among the Solanaceae members like *Withania somnifera* (Davis, 1966) [17]. The basic type of wall development in the present study comprises of persistent single layered epidermis, an endothecium, two middle layers and bilayered glandular tapetum initially.

A multilayered endothecium has been observed in some Solanaceae such as *Nicotiana glutinosa* and *Nicotiana tobaccum* (Jose and Singh, 1968) [32] and in the present study single layered endothecium is found without fibrous bands of thickenings and the same has been recorded in *Withania somnifera* (Balakrishna G. and Kweon H. 2012) [9]. But in *Solanum nigrum* a fibrous thickening of endothecium was found only at the anther tips (Saxena and Singh, 1969) [53]. In the present study the fibrous bands of thickenings in the endothecium is not observed but the stomium starts exerting the pressure inside due to loss of water by the cells of endothecium, with the results the stomium rupture and the anther dehisces.

In the present study tapetal cells are glandular, in *Withania somnifera*. (Davis, 1966) [17] also. Tapetal cells become dimorphic, binucleate and dual in origin and the same has been recorded in *Mimulus ringens* (Arekal, 1965) [8] and *Mimulus guttatus* (Urs and Jayaraj, 1997) [60] and *Alectra thomsoni* (Vijayaraghavan and Ratnaparkhi, 1973) [61] of the different family. Both amoeboid and glandular types of tapetal cells have been observed in *Datura stramonium* (O' Neal, 1920) [44] the former being common. The number of middle layers varies, in most of the members of Solanaceae, and during the anther dehiscence the middle layers helps in the anther dehiscence.

In the present investigation the one middle layer is persistent until maturation of pollen grains. They undergo considerable stretching to keep pace with the developing sporangium and finally becomes crushed and obliterated, soon after the formation of microspores. As the cells of middle layers lack the ability to divide anticlinally, the tissue cannot adjust itself to the pressure exerted by the multiplying and expanding sporogenous cells within each sporangium. The cells of the middle layer ultimately become flattened and crushed at the time of meiotic division in the pollen mother cells; the same has been recorded in *Penstemon nitidus* (Jayaraj, 2003) [31]. In many species, the cells of middle layers of starch and other reserves which get mobilized during later development of pollen.

The tapetum undergoes nuclear division as the nutritional requirements are increased usually during the meiosis. The tapetal cells are binucleate in the present study and *Withania* has 2, *Capsicum* has 3, and *Atropa* has 4 nuclei (Olmstead *et. al.,* 2008) [45] in tapetal cells and this is the most inconsistent feature of the anther and male gametophyte in the number of nuclei in tapetal cells (Tobe, 1989) [59].

Meiosis in microspore mother cells accompanied by simultaneous cytokinesis and resulting microspore tetrads are predominantly tetrahedral.

In the present study the primary sporogenous layer undergoes mitotic division and form a mass of sporogenous tissue and further differentiates into pollen mother cells, which further starts separating and forms into an meiocyte with conspicuous nuclei and thick wall around which callose is surrounded and this covering of callose (Heslop-Harrison,1964) [29] around the microspore / pollen mother cells is considered to isolate the meiocyte from the surrounding diploid tissue to achieve nuclear independence. These pollen mother cells undergoes meiosis and forms dyad and tetrahedral tetrads. The uninucleate microspores are released from the tetrad, the exine and intine formation takes place. At maturity pollen grains are two celled in the present study being common in Solanaceae members (Davis, 1966) [17].

In general correlation of growth patterns of anther tissues appears to be simple when viewed morphologically. The metabolic substances play an important role during in the revolutionization of our knowledge about structural and functional aspects of anther. Such information is of great significance because at each and every step of cell / tissue /organ differentiation there is a direct involvement of metabolites.

In the present study the primordium has undifferentiated mass of cells and it devoid of starch grains and it is not reported in other species.

Characteristic of sporogenous tissue is lack of starch granules. However, the tissue in *Euphorbia* (Rudramuniyappa and Annigeri, 1985) [51] and *Calanthe masuca* (Hegde and Rudramuniyappa, 1984) [28] shows synthesis of storage carbohydrates, visible in abundance in the form storage granules. Starch grains in the microspores and pollen grains presumably are concerned with the availability of energy, required for pollen viability and germination (Malik, Bhattacharya and Singh, 1978 [40]; Andrade and Hegde, 1983 [7]; Rudramuniyappa and Annigeri, 1985) [51]. In many species, the cells of the middle wall layers and storage centers of starch and other reserves which gets mobilized during later development of the pollen (Jayaraj, 2003) [31].

The sporogenous tissues are thin walled and show moderate amount of carbohydrate distribution, the same has been recorded in *Euphorbia* (Rudramuniyappa and Annigreri, 1985) [51]. In the present study the sporogenous tissue shows nutritional correlation between the sporogenous tissue and the surrounding anther tissues, and it said that the cellular interaction between the surrounding sporophytic tissue and reproductive cells is a key requirement for the normal development of anther.

In the meiocyte stage, the meiocyte and all the wall layers except the tapetum are rich in carbohydrate and also show abundant starch grain deposition in the connective and wall layers. And this shows that with the growth and development of the sporogenous cells, the high intensification or concentration of starch grains are seen in epidermis, endothecium, middle layer, partition wall and connective. Synthesis of callose, inside primary walls of meiocytes is a common features of in the angiosperm anthers and the same has been seen in many plants and callose is rich (Bhandari and Sharma, 1983; Katti, *et al*. 1994; Vijayaraghavan and Sudesh, 1994) [13, 35, 62].

According to Noher de Halac *et al.* (1990) [43] accumulation of starch in storage tissue is an indication of development of

metabolism in the sporogenous cells. Gradual reduction in the starch grains from the storage tissue on one hand and progressive growth and differentiation of sporogenous cells on the other hand suggests the existence of nutritional correlation between them.

In the present study, at the completion of meiosis, the carbohydrate content increases in tetrads and the microspores are set free by the dissolution of callose wall around the tetrad. After formation of dyad and tetrads the concentration of starch is restricted to wall layers. At this stage there is no decrease in carbohydrate (Present study). It is also same in the case of *Calanthe masuca* (Hegde and Rudramuniyappa, 1986) [28], *Lilium* (Wilson and Dickinson, 1983) [65].

Newly released microspores are rich in carbohydrate. In the present study the presence of rich content of macromolecules indicates that the increase in volume of microspores is accompanied by increase in cytoplasmic content. The uninucleate microspores released from the tetrad synthesize exine and intine. At the maturity the pollen grains are two celled in the present study being common in Solanaceae (Davis 1966) [17]. In the present study the pollen grains shows degenerating tapetum rich in the amount of carbohydrate and same has been recorded in *Calanthe masuca* (Hegde and Rudramuniyappa, 1986) [28].

6. Conclusion

The present ontological study on the developing anthers of *Solanum viarum*, Dunal is an attempt to identify any peculiar feature(s) associated with microsporogenesis. Anther is tetrasporangiate, wall development follows basic type. Epidermis and endothecium is single layered and persistent. Middle layers are two in number. Tapetum is binucleate glandular, dimorphic in nature, dual in origin (i.e., p-type & c-type).

Nutritional correlation between sporogenous tissue and surrounding anther tissues is well established that, the cellular interaction between surrounding sporophytic tissue and reproductive cells is a key requirement of the normal development of anther. Maturity of the microspore leads to degeneration of tapetal cells. A correlation can be observed between degeneration of tapetum with microspore development indicates it is a nutritive in function. Microspore tetrads are tetrahedral, pollen is two-celled and triaperturate with exine and intine. So it can be concluded that there is mutual interaction and utilization of biochemical substances in the anthers during formation and differentiation of pollen grains.

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