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RESEARCH ARTICLE

DISTRIBUTIONAL PATTERN OF RNA IN *SOLANUM VIARUM*, DUNA L.

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ABSTRACT

Considerable histological information on anther development was done by many scientists, but histochemical information on the anther development of Solanaceae members is almost lacking. An attempt is made to understand the distribution of histochemical substances during anther development in *Solanum viarum*. In the present study anther wall development is basic type, epidermis and endothecium is single layered and persistent. Middle wall layers are two in number, tapetum is binucleate glandular, dimorphic in nature, dual in origin. Rich content of RNA is seen in archesporial cells, sporogenous tissue, meiocyte, tapetal cells, wall layers and pollen grains and connective. 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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INTRODUCTION

In recent years considerable work has been done on the anther histochemistry (Maheshwari, 1950; Heslop-Harrison, 1972; Mascarenhas, 1975 Vithange and Knox, 1979, 1980; Rudramuniyappa and Annigeri, 1984, 1985; Bhandari and Sharma 1983; Bhandari 1984; Raghavan, 1997; Agadi S.N. 2012, 2014a 2014b, 2014c, 2016) To learn more 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). 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 sporogenesis 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 Mascarenhas,1989) are formed which are differ in cellular contents that can be termed as cytoplasmic heterospermy. 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).The anther wall is formed by specific number of cell layers that originate in the earliest developmental stages. Davis (1966) observed Four types of anther wall development, 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 another development, such the Commelinaceae having type I and type III (Hardy, *et al.,* 2000).And the family Solanaceae having type I type II (Carolina Carrizo Garcia, 2002). The presence of a callose wall around meiocytes 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). 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 Majewska- Sawka, 2011). Following degradation of the callosic tetrad walls, the microspores are released into mature pollen grains (Wan, *et. al.* 2011; Xie, *et. al.*2010). 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) in *Solanum muricatum* and Young (1922) in *S. tuberosum*

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invoked the Lilium type, and this was criticized later by Bhaduri (1932), who reported Polygonum-type development in *S.melongena*. Cooper (1931), Bhaduri (1932, 1935), Satina (1945), Goodspeed (1947), Parashar and Singh (1986), Villari and Messina (1996), Karihaloo and Malik (1996), Carrizo Garcia (2002) and others made significant contributions to the embryology of a variety of Solanaceae species. To understand the biochemical language which acts as a stimulus for establishment of organogenesis from simple to complex, histochemistry can be used as a tool. Application of histochemical method has enabled the localization of some chemical substance which have contributory role in growth and development of *solanum viarum* characters. Although there are some excellent papers on the ontogeny of solanaceae members, which contain a great deal of information on structural changes but no one has attempted to analyze the histochemical and embryological basis on another development in *solanum viarum* so present work has been taken.

Aims and objectives

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

- To characterize the peculiarities of the reproductive structure.
- To know the histochemical localization of RNA at different development stages of anther.

MATERIALS AND METHODS

The flower buds of different development stages of *Solanum viarum* were collected from Botanical garden, Karnatak University, Dharwad and fixed in the (FAA). Employing standard histochemical procedure, 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 microtome. In the present investigation following method is employed to localize metabolite.

Localization of RNA with Toludine blue method (Chayen *et al.*, 1973)

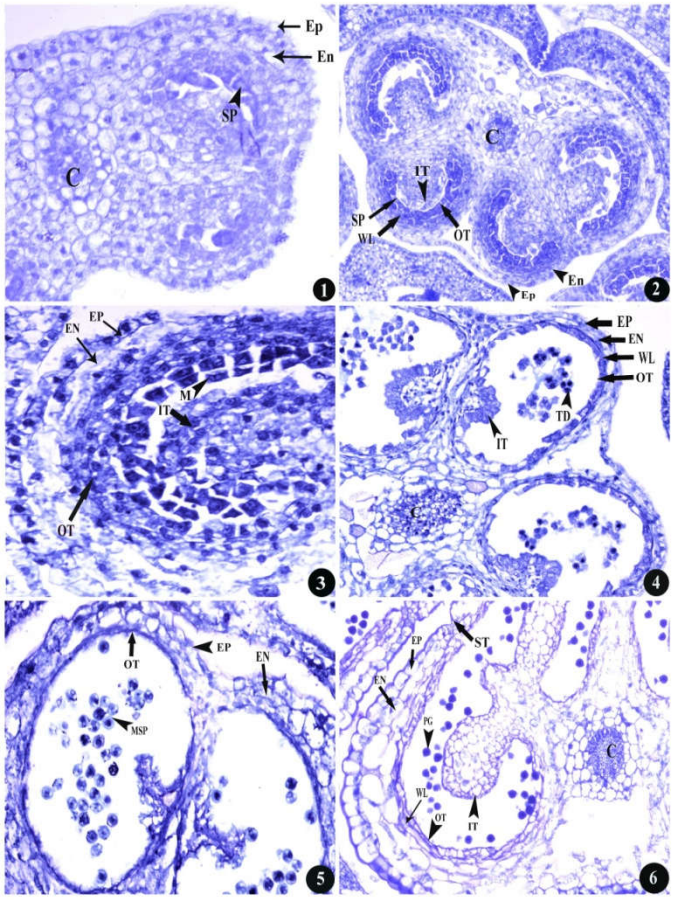
Total insoluble RNA is localized by employing Toludine blue method. In this method, phosphoric acid is responsible for staining RNA with toludine blue. This technique is based on phenomenon of metachromasia in which certain cell constituents stain differently from the original colour of dye. The purple colour of the dye is termed as orthochromatic shade and blue is called as metachromatic shade. Although toludine blue stains nucleic acid, only the localization of RNA is taken into account in the present investigation.

Staining procedure

- Sections were deparaffinised in xylene and hydrated sections were incubated in 0.05%
- Toludine blue for 5 minutes at room temperature.
- Rinse the slides in distilled water for 5 minutes.
- Air dry the slides, cleared in xylene.
- Mount in DPX and observe the slides under Microscope for localization.

Colour indication: RNA stains purple in colour.

PLATE



Abbreviations: EP=Epidermis; EN=Endothecium; ML=Middle Layers; T=Tapetum; SP=Sporogenous; M=Meiocyte; TD=Tetrad; MSP=Microspores; PG=Pollen grains; ST=Stomium; C=Connective.

Plate. Transverse section of *Solanum viarum*, Dunal. another tested for RNA.

- Four lobed anthers with archesporial cells rich in RNA.
- Tetrasporangiate anther showing rich content of RNA in the sporogenous tissue and connective. Whereas wall layers show less amount of RNA.
- Sporangium showing rich amount of RNA in meiocytes and tapetum.
- Tetrahedral tetrads, tapetum and connective showing rich content of RNA.
- The tetrasporangiate anther with degenerating tapetum and microspores possess rich content of RNA.
- Matured pollen grains are rich in RNA. It's also showing stomium.

Observations

Distributional Pattren of Total RNA (Fig 1-6).

The four lobed structures show uniform distribution of RNA in hypodermal archesporial cells. The connective cells are rich in RNA than the surrounding cells (Fig. 1). The sporogenous tissue which is derived from the archesporium also retains high concentration of RNA. The sporogenous cells and middle wall layers and connective show more RNA than the epidermis, endothecium middle wall layers and tapetum (Fig. 2). The fully differentiated meiocytes and tapetum show high amount of RNA. However, at the time of meiosis the concentration of

cytoplasmic RNA slightly decreases in the meiocytes, and not in the tapetum (Fig. 3). At the completion of meiosis, the RNA content increases in the tetrahedral tetrads. At this stage tapetum and connective also shows rich cytoplasmic RNA (Fig. 4). The vacuolated microspores shows moderate amount of RNA (Fig. 5). At this stage less amount of RNA is found in middle layer, epidermis and endothecium and degenerating tapetum. At maturity, pollen grains are rich in RNA and connective also shows a rich deposition of RNA (Fig. 6).

DISCUSSION

RNA and protein biosynthesis is activated by Ascorbic acid, which in turn accelerate the cell division and differentiation (Chinoy *et al.,* 1971). 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 reaches maturity. 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. By adopting histochemical technique it is possible to acquire body information on both structural and chemical composition of cells and tissues. Scientists have given considerable histochemical information 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). Katti, *et.al,* 1994; Hegde and Isaacs, 1992; Agadi, 1996, 2012, 2014a, 2014b, 2014c, 2016; Jayaraj, M. 2003). Majority of solanaceae members shows, anther is tetrasporangiate. In this wall layers development is basic type (Davis, 1966). *Datura* is exceptional only one species was included in basic type, *Datura ceratocaula*, (Carrizo Garcia, 1998). In contrast to Davis' suggestion (1966), 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 *Atropa belladonna*, (Prakash, 1987; Sharma *et,al.,* 1987) W*ithania somnifera* (Davis, 1966) *Solanum glaucophyllum* (Carolina Carrizo Garcia, 2002).

The present anther developmental 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* (Carrizo Garcia, 1998 and Thiagarajan, 1986), respectively. But the basic type of anther wall formation is considered as an exception among the solanaceae members like *Withania somnifera* (Davis, 1966). 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. In the present study single layered endothecium is found without fibrous band of thickenings and the same has been recorded in *Withinia somnifera* (Balakrishna, G. and Kweon, H. 2012). But in *Solanum nigrum* a fibrous thickening of endothecium was found only at the anther tips (Saxena and Singh, 1969). 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. The tapetal cells become dimorphic, binucleate and dual in origin and the same has been recorded in *Mimulus ringens* (Arekal, 1965) and *Mimulus guttatus* (Urs and Jayaraj, 1997) and *Alectra thomsoni* (Vijayaraghavan and Ratnaparkhi, 1973) of the different family. Both amoeboid and glandular types of tapetal cells have been observed in *Datura stramonium* (O' Neal, 1920) 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). 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) 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). In the present study meiosis in microspore mother cells is simultaneous cytokinesis and resulting tetrahedral tetrads are formed. 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) 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). In the present study the uniform distribution of RNA in the cells of anther primodium, only the hypodermal cells at four corners of primodium enlarge and differentiate into archesporial cells. This specific positional establishment of archesporial cells is presumed to be due to differential distribution of auxin (Scott *et al.,* 2004). The sporogenous tissues are thin walled and show moderate amount of insoluble RNA distribution, the same has been recorded in *Euphorbia* (Rudramuniyappa and Annigreri, 1985). 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 present study, at the completion of meiosis, the RNA content increases in microspore tetrads and the microspores are set free by the dissolution of callose wall around the tetrad and shows rich content of RNA, the same has been reported in *Kalanche,* (Rudramuniyappa *et al.,* 1984). After formation of dyad and tetrads the concentration of starch is restricted to wall layers. At this stage there is no decrease in RNA (Present study). It is also same in the case of *Calanthe masuca* (Hegde and Rudramuniyappa, 1986), *Lilium* (Wilson and Dickinson, 1983). Newly released microspores are rich in RNA. 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). In the present study the pollen grains shows degenerating tapetum rich in the amount of RNA and same has been recorded in *Calanthe masuca* (Hegde and Rudramuniyappa, 1986). Pollen grains are richly stained with RNA. At the dehiscence stage except connective and pollen grains RNA content is decreases.

Conclusion

The present ontological and histochemical study on the developing anthers of *Solanum viarum* is an attempt to identify any peculiar feature(s) associated with microsporogenesis and histochemistry reveals the following features. Another is tetrasporangiate, wall development follows basic type. Epidermis and endothecium is single layered and persistent. Middle layers are two in number. Tapetum is binucliate glandular, dimorphic in nature, dual in origin (i.e., p-type & c-type). 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. Rich content of RNA is present in archesporial, connective, sporogenous, meiocytes and tapetal cells, wall layers and pollen grains. In sporogenous stage rich content of Starch granules in the wall layers, partition wall and connective is observed. Sporogenous cells acquire rich histochemical contents because of nutritional correlation between them and surrounding anther tissues. It is well established that the cellular interaction between surrounding sporophytic tissue and reproductive cells is a key requirement of the normal development of anther. In *solanum viarum*, glandular, binucleated, dimorphic tapetum show rich content of RNA. By the completion of meiosis the tetrads shows increase in RNA content. This indicates that tapetum constitutes a tissue specialised for storing and supplying basic nutritive substances for developing pollen grains. The microspores are released from tetrads by the dissolution of callose and shows rich content of RNA. Further these microspores undergo gametogenesis and show rich accumulation of RNA deposition, synthesis and degradation of RNA is observed at specific stages of anther development. So it can be concluded that there is mutual interaction and utilisation of biochemical substances in the anthers during formation and differentiation of pollen grains.

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