Ontogeny and Histochemistry of anther development in *Russelia equisetiformis* Schlecht & Chan.

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ABSTRACT

As part of an overall program aimed at increasing our knowledge of male reproductive system of *Russelia equisetiformis*, this study documents structural developmental and distributional pattern of insoluble polysaccharide, protein, RNA and to correlate this with corresponding growth phase of the anther. The Anther and pollen development were studied with light microscopy after histochemical staining. Result showed that some ultra-structural characters of anther and pollen grains. Anther is tetrasporangiate, epidermis and endothecium is single layered and develops fibrous thickenings, middle wall layers are bilayered, tapetum is glandular, vacuolated dual in origin, and bilayered (at certain regions). Meiocytes undergo meiotic division producing tetrads, encased within callose wall. After the microspore development tapetum degenerates, but the exact phase of degeneration is not determined. After dissolution of callose wall, young microspores grow rapidly thus exine wall is synthesised. Young microspores grow into pollen grains which accumulate starch. Histochemical localization show positive results during developmental stages.

Keywords: *Russelia equisetiformis*, anthers, pollen, tapetum, polysaccharide, protein, RNA

INTRODUCTION

Development is an orderly unfolding mechanism during which different parts as well as various physiological activities are correlated with the rest resulting in the formation of an integrated organised system. Microsporogenesis is a paradigm of growth and development in which all the developmental phenomena are displayed. 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. The anther consists of sporophytically derived tissues which nourish and protect the male gametophyte. The developmental events in the anther reveal that pollen formation involves the coordinated functioning of diploid sporophytic and haploid gametophytic tissues. Each growth phase of an anther is characterized by the synthesis of certain macromolecules, soluble metabolites and enzymes. The present paper is an attempt to recognise the various morphogenetic phenomena that occur during microsporogenesis and localization of histochemical substances within cells and tissues of anther.

MATERIALS AND METHODS

The different developmental stages of flower buds of *Russelia equisetiformis* were collected from the Department of Botany, Karnataka Science College, Dharwad and were fixed in FAA for 12 hrs. Collected flower buds were dehydrated, infiltrated, and embedded in paraffin wax. 6µm thick sec-

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tions of anthers were cut and processed for the staining. The different localization of substances are done for insoluble polysaccharides (PAS Method), RNA (Toludine Blue Method) and proteins (Amido-black 10B Method) [1]. The results were recorded in the microphotographs and expressed as 'rich' or 'low 'etc, arbitrarily to denote visual intensities of stain in the observation.

RESULTS AND DISCUSSION

Anther development involves cascade of events, beginning with differentiation of archesporial cells in the hypodermal region. The large archesporial cells are thin walled and free from storage carbohydrates. Periclinal division of archesporial cells leads to the differentiation of outer primary parietal layer and inner primary sporogenous layers. Two or more periclinal divisions in the primary parietal cells produces an endothecium, two middle wall layers and bilayered glandular tapetum at certain place. The primary sporogenous layer divides in all possible planes to produce mass of sporogenous cells. The sporogenous cells are characterised by inconspicuous PAS-positive walls (Plate Ia). Sporogenous cells divide actively and their nuclei become conspicuous (Plate Ia). Concurrently, cell walls of sporogenous cells become thin walled and less in polysaccharides (Plate Ia), but sporogenous cells are devoid of starch storage. Sporogenous cells are compactly arranged without any intercellular spaces (Plate Ia). The walls of meiocytes undergoes hydrolysis, prior to meiosis, meiocytes increases in size, meiocytes round off and contain rich polysaccharides in cytoplasm. The nucleus enlarges in meiocytes. During initiation of meiosis, the condensed chromatin of meiocytes nuclei is arranged periphery, meiocytes lack additional wall deposition.

First meiotic division forms dyad, is very conspicuous and PAS-positive. Dyads lack storage carbohydrates and cytoplasm shows moderate staining. During meiosis, tapetum shows dimorphism and rich in polysaccharides. The completion of second meiotic division results in the formation of microspore tetrads which are arranged in isobilateral fashion (Plate Ib). Tetrads with rich content of polysaccharides and less stained callose wall (Plate Ib). During tetrad formation the tapetal cells degenerate, even though they are rich in polysaccharides. After complete degeneration of tapetum, fibrous bands of thickenings are developed in endothecium, which even spreads towards connective tissue and in this stage free microspores show rich content of polysaccharides (Plate Ic). Mature pollen grains show tricolporate nature and the exine are rich in polysaccharides (Plate Id).

The anther primordium is having rich content of protein. High amount of protein is observed in epidermis, endothecium, middle wall layers and tapetum, which anther wall are clearly distinguished and differentiated (Plate Ie). Subsequently, rich content of protein sustain only in sporogenous cells and tapetum, whereas protein declines in the parietal layer. In meiocytes nuclei stains darken than cytoplasm. Glandular, bilayered vacuolated tapetal cells are rich in protein content compared to other wall layers. Tetrads, fibrous thickening and pollen grains are rich in protein (Plate If,g,h,i respectively).

The young anther primordium contains a uniformly high amount of RNA. Sporogenous tissue contains rich amount of RNA (Plate Ii). The cytoplasmic RNA is very low in the connective tissue. At meiocyte stage nuclei is darker than cytoplasm, rich in RNA. Tapetum, tetrads (Plate Ij) and pollen grains (Plate Ik,l) are also rich in RNA.

The role of pollen grains as a male partner in sexual reproduction of seed plants was established by the end of the 19th century [2]. It is in the anther, the fertile part of the stamen, microsporogenesis and pollen grain formation occurs. The anther is specialised heterogeneous cell system and is involved in the protection, nutrition and dispersal of pollen. Histochemistry enables identification and localization of synthesis, storage, transport and metabolism of the tissue concerned. As in majority of taxa of Scrophulariaceae the anther in *R. equisetiformis* is tetrasporangiate. The development of microsporangium wall conforms to the basic type [3]. Nevertheless, a Monocotyledonous and basic type of wall development have been observed in *Striga euphrasiides*, *S. orobanchoides* [4] and

Alectra thomsonii [5]. But in majority of the members of family, it is dicotyledonous type. The basic type of wall development in the present study comprises of persistent single layered epidermis, an endothecium, two middle wall layers and bilayered glandular tapetum (at certain places).

In the present study, it is observed that the cells of the endothecial layer elongate slightly radially before developing the characteristic fibrous bands of thickenings as in other investigated taxa of Scrophulariaceae. In the present investigation, it is also observed that the fibrous bands of thickenings in the endothecium differentiate only after complete dissolution of tapetum as seen in most of the investigated taxa of angiosperms. This indicates that the tapetum inhibits the development of fibrous bands of thickenings in the endothecium [6] as long as it is functioning. The numbers of middle wall layers vary; in most of the members of Scrophulariaceae only one middle layer have been recorded in the microsporangium wall, but in the present investigation the middle layer undergoes periclinal divisions forming two middle wall layers which remain persistent even at pollen shedding stage, a feature also observed in *Penstemon nitidus* [7] and *Chelone glabra* [8] and other few member of this family. In many species, the cells of the middle wall layers, an storage centres of starch and other reserves which get mobilized during later development of pollen [7].

The glandular tapetum is bilayered at certain places and exhibits tapetal dimorphism as observed in *Mimulus ringens* [8] and *Mimulus guttatus* [9] and *Alectra thomsoni* [5] of the same family. Present study showed tetrahedral and isobilateral type of tetrads. However, in *Penstemon nitidus* [7] and *Adenosma bilabiatum* [10] tetrahedral tetrads are recorded. Where as in *Melampyrum pratense* [11] isobilateral tetrads are recorded. In the present investigation pollen grains are two celled at the time of anther dehiscence and are triaperturate, the similar feature is recorded for the member of the family, viz. *Adenosma bilabiatum* [10] and *Penstemon nitidus* [7] and many taxa of angiosperms.

In the present study during development, uniform distribution of polysaccharides, Protein and RNA [12,13]. As the development of anther progress, sporogenous tissue with less content of starch is observed and is also reported in *Morus alba* [14] but in *Clerodendrum serratum* [15]. It is exceptional case where starch is present, same has been recorded in *Saxifraga ciliate* [16], *Calanthe masuca* [17]. High protein content is seen in sporogenous tissue and wall layers in the present study [15,18-20]. Later stage of PMC's which are vacuolated attains maximum polysaccharides, protein and RNA content [15-17,21,22]. In the present study presence of polysaccharides, RNA and protein is observed. This indicates that tapetum constitutes a tissue specialised for storing and supplying basic nutritive substances for developing pollen grains. However, same has been reported in the tapetum in *Kalonchoe* and *Euphorbia* [21,23]. But it is exceptional in *Calanthe masuca* [17], where tapetum is all through its life does not store insoluble polysaccharides.

In the present study, fully differentiated meiocytes and glandular bilayered tapetum show rich protein and RNA content. It is also reported in *Saxifraga ciliata* [16] and *Clerodendrum serratum* [15]. This indicates that tapetum acquires metabolic hyperactivity at the time of meiosis. At the completion of meiosis the starch content increases in microspore tetrads and pollen grains [21] and also reoccurrence of protein in the present study. Rich cytoplasmic RNA content in tetrads, tapetum and callose wall around the tetrads. In the present investigation starch grains are found in endothecium, which is utilized for the formation of endothecium and also thickenings of the wall [16,17,21]. The newly released microspores are rich in RNA and it is also true with *Zea mays* [24], *Tamarix troupii* [25], *Clerodendrum serratum* [15], *Potamogeton richardsonii* and *Eichhornia crassipes* [26]. However, in *Najas marina* [27] the microspore tetrads show less content of RNA. The degenerating tapetum shows a high amount of RNA [28]. Pollen grains show rich content of insoluble polysaccharides in the present study and the same is observed in *Calanthe masuca* [17,29].

The results obtained in the present study have shown that the anther of *R. equisetiformis* shows following features. Anther is tetrasporangiate, wall development follows basic type, epidermis is single layered and persistent. Endothecium is single layered and develops fibrous bands of thicken-

ings. Middle wall layers are two in number. Glandular tapetum is dual in origin, vacuolated and bi-layered (at certain regions). After the microspore development tapetum degenerates but the exact phase of degeneration is not determined. A correlation has been observed between degenerating tapetum and differentiation of fibrous bands of thickenings in endothecium. This indicates that the presence of tapetum has inhibitory influence on the differentiation of endothecial thickenings. Degeneration of tapetum during microspore development indicates that it is a nutritive tissue. Microspore tetrads are tetrahedral and isobilateral, mature pollen is two celled, triaperturate or trisyncalporate and shows exine and intine.

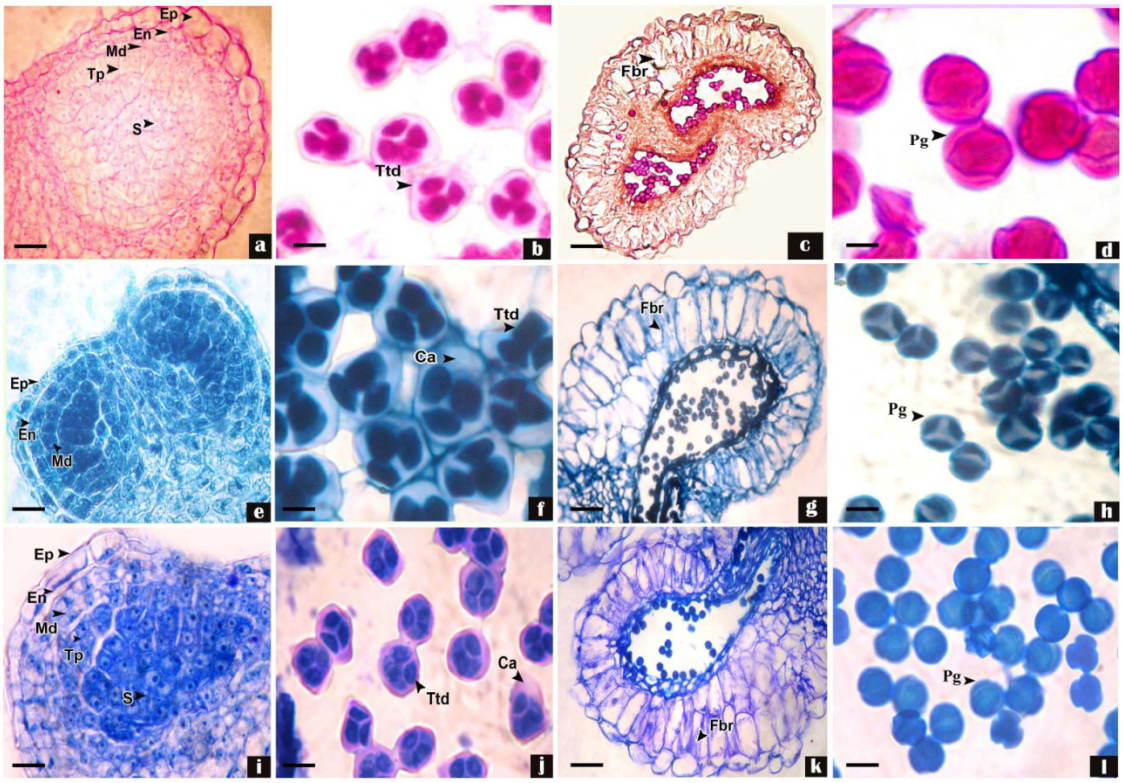

Plate I. TS of anthers development in *R. equisetiformis*: (a) PAS-test for total insoluble polysaccharides-anther locule showing less content of polysaccharides in sporogenous tissue and wall layers (20X × 4.3X); (b) Tetrads with very rich content of polysaccharides and less stained callose wall (40X × 6.7X); (c) Endothecial wall showing fibrous thickening with rich polysaccharides content; (d) Tricolporate nature of the pollen grain which are darkly stained with polysaccharides (10X ×100X); (e) Tested for total proteins-anther showing wall layers differentiated into outer epidermis followed by an endothecium, middle layer, tapetum and sporogenous tissue with rich protein content (100X × 4.3X); (f) Tetrahedral tetrads are surrounded by callose wall showing rich content of protein (100X × 4.3X); (g) Magnified sporangium with darkly stained free pollen grains and fibrous bands of thickenings in endothecium (40X × 6.7X); (h) Mature triaperture or tricolporate pollen grains (Trisyncalporate nature) with rich content of protein (10X × 100X); (i) Tested for RNA-anther locule showing sporogenous tissue with rich content of RNA (100X × 4.3X); (j) Tetrahedral tetrads surrounded by callose wall showing rich content of RNA (40X × 6.7X); (k) Anther locule showing fibrous bands of thickenings in endothecium with remnants of bilayered tapetum with rich content of RNA (40X × 4.3X); (l) Mature pollen grains which are darkly stained for RNA (10X × 100X).

Rich content of polysaccharides, protein and RNA is present in sporogenous cells and tapetum. During early stages, polysaccharide content is less in sporogenous tissue but in later stages of PMC's which are vacuolated attains maximum polysaccharides content and is reported for few species. Presence of polysaccharides, RNA, and protein in Glandular bilayered, vacuolated tapetum of *R. equisetiformis* has been observed. This indicates that tapetum constitutes a tissue specialized for storing and supplying basic nutritive substances for developing pollen grains. By the completion of meiosis, the tetrads which are isobilateral and tetrahedral shows increase in content of polysaccharides, RNA and protein. The endothecium of *R. equisetiformis* develops PAS-positive, fibrous thickenings and the stored starch is utilized for the formation of endothecial thickening and in the same way protein and RNA also serves as a storage products. Pollen grains show triaperturate/trisyncalporate nature and contains rich amount of polysaccharides, RNA and protein. Synthesis and degradation of polysaccharide, protein and RNA is observed at specific stages of anther development. So it can be concluded that there is a mutual interaction and utilization of biochemical substances in the anther during formation and differentiation of pollen grains.

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