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CYTOCHEMISTRY OF MEIOCYTES, MICROSPORES, POLLEN AND TAPETUM IN *PSOPHOCARPUS TETRAGONOLOBUS* (L) DC

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In the present work stress is given more on the cytochemistry of developing sporogenous cells, meiocytes, microspores, pollen and the tapetum in anthers of *Psophocarpus tetragonolobus* (L) DC. RNA and protein are high in the sporogenous cells, decline slightly during meiosis I and increase rapidly in the spore tetrads. The concentration of RNA and protein varied within and among the spore tetrads. Synthesis of insoluble carbohydrate occurs at the meiocytic stage and subsequently increases during its growth. The additional wall thickening around the meiocytes and tetrads gave a strong pink colour with the PAS test. Initiation of the sporogenous cells is followed by the synthesis of ascorbic acid in the cytoplasm. A high quantity of ascorbic acid is noted in the meiocytes, spore tetrads, microspores and pollen. Maturation of microspores is associated with high accumulation of RNA, protein, carbohydrates and ascorbic acid. The exine of mature pollen reacts with Azure-B and shows a faint green colour. Presence of high quantity of RNA, protein, carbohydrates and ascorbic acid in the tapetal cells from the meiocyte stage onwads reveals that the tapetum is a specialised tissue meant for storing and supplying basic nutritive substances to the developing pollen.

Keywords : *Psophocarpus tetragonolobus* (L) DC; Meiocytes; Microspores; Tapetum; RNA; Protein; Insoluble carbohydrate; Ascorbic acid.

Introduction

The winged bean, *Psophocarpus tetragonolobus* has attracted worldwide attention due to its high nutritional value and multiplicity of uses. The International Grain Legume Information Centre (1978) has reviewed its Morphology, Physiology, Cytology, Genetics, Breeding and Taxonomy, but not the Reproductive Biology. Hence, in the present study a part of Reproductive Biology of *Psophocarpus tetragonolobus*, the *in situ* changes of physiolgically active substances namely RNA, protein, insoluble carbohydrates and ascorbic acid in the developing sporogenous cells, their derivatives, and the tapetum is taken up. The physiological, biochemical and histochemical changes in the anther have received great attention of Kamizyo and Tanaka (1982). Periodic synthesis of RNA occurs during microsporogenesis with its peak during the premeiotic interphase (Dickinson and Heslop-Harrison, 1970). The insoluble polysaccharides in the anther cells of *Oryza sativa* (Jain 1981) are low in the archesporium and pollen mother cells but increases during the meiotic microspores. Protein synthesis undergoes a stage specific variation during meiosis and pollen development. Apart from the nutritive role, the tapetum synthesizes orbicules of sporopollenin nature (Echlin, 1971; Kapil and Tiweri, 1978) and substances which participate in the incompatibility system (Nettancourt, 1977). The present investigation is to understand the physiological relationship between the tapetum and the developing pollen.

Materials and Methods

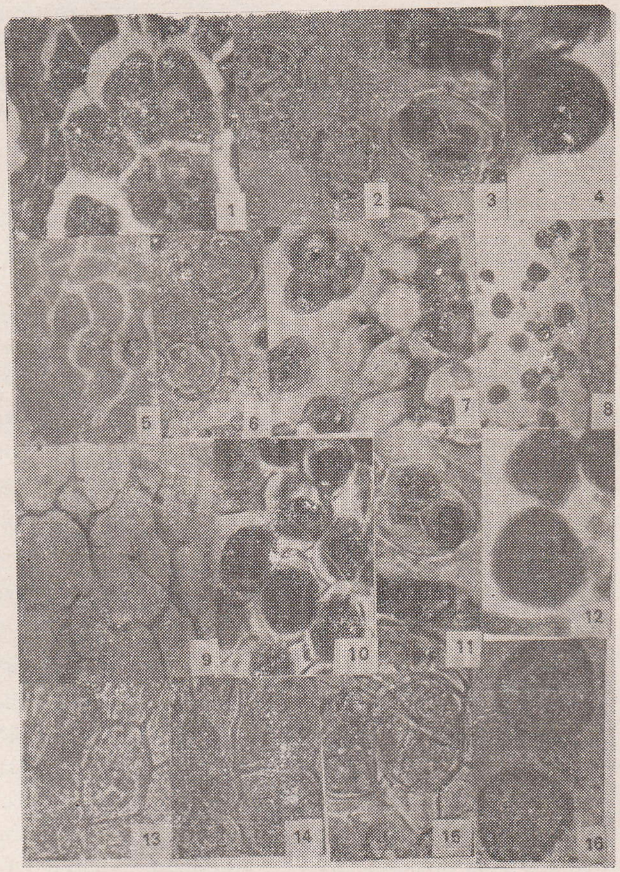
Different sizes of flower buds were fixed in formalin-acetic-alcohol and Carnoy's fixative. The material was processed by employing standard microtechnique procedures for dehydration, paraffin infiltration and embedding. The paraffin material was cut at 8 μm thickness and the sections were mounted onto slides by using gelatin adhesive. The following standard histochemical methods were used to localize the RNA-Azure-B (Jenson, 1962); total proteins-mercuric bromophenol (Mazia *et al.*, 1953); insoluble carbohydrates-PAS method (Jensen, 1962) and Ascorbic acid-acidified alcoholic silver nitrate (Patel *et al.*, 1976). Adequate controls were also run.

Observations

RNA—The early sporogenous tissue shows a high amount of RNA in the cytoplasm (Fig. 1). When it develops and differentiates into meiocytes, the substance declines in the cytoplasm (Fig. 2). The additional thickening around the meiocytes does not react with Azure-B. In the tetrad, the microspores show slightly increased RNA content in the cytoplasm. The concentration of RNA varies within and among the spore tetrads (Fig. 3). In some tetrads, only one or two spores show a high RNA content. As the microspores matures, the RNA increases to a higher level (Fig. 4). In the mature pollen, the exine reacts with Azure-B stain and gives a faint green colour.

The tapetum is quite rich in RNA at its initiation (Fig. 1). At the meiocyte stage, the tapetum show a high RNA content. The tapetal cells are vacuolate and the nucleoli very rich in RNA. The higher RNA content is maintained until its degeneration (Fig. 3). Throughout the ontogeny of the tapetum, the cell walls react negatively to the Azure-B test.

Protein—The sporogenous cells have high protein content, but the cell walls are faintly protein positive (Fig. 5). The reduced level of protein content in the PMCs at prophase shows slight increase on attaining dyad and tetrad stages (Fig. 6 and 7). The additional wall thickening around the meiocytes and tetrads does not react with protein stain. A gradual increase of protein in the cytoplasm of the microspores shows further increase in the mature pollen (Fig. 8).



Figs. 1-16 Anther sections of *Psophocarpus tetragonolobus* (L) DC.
1-4 Cells tested for RNA (X624, X120, X650 & X 572);
5-8 Cells tested for Protein (X780, X90, X650 & X162);
9-12 Cells tested for Carbohydrates (X572, X572, X650 & X520); 13-16 Cells tested for Ascorbic acid (X650, X585, X650 & X650).

The protein content of the tapetal cells from the meiocyte stage is maintained at a high level till the degeneration of the cells. The cell walls however react negatively to the Azure-B test.

Carbohydrates—The feeble expression of PAS stain in the cytoplasm of the sporogenous cells (Fig. 9) gradually increases with the developmental stages. At prophase, the meiocytes develop additional PAS positive wall thickenings (Fig. 10). There was a little increase in the cytoplasm of the spore tetrad (Fig. 11). The spore walls and the additional wall thickening are distinctly PAS positive. In the young microspores, the wall is PAS positive and cytoplasm shows a moderate pink-colour. The mature pollen exhibits abundant PAS positive tinge (Fig. 12).

The glandular tapetum, to begin with, is completely devoid of PAS positive substances. It is initiated at the commencement of prophase in meiocytes. An increase in the quantity of PAS positive tinge and its accumulation persists till the completion of meiosis. During meiosis the cells are highly vesiculated and cell walls are distinctly PAS positive. The degenerating tapetum is devoid of starch, but takes PAS positive stain.

Ascorbic acid—Synthesis of ascorbic acid occurs in the sporogenous tissue, and further increase and accumulation si maintained with the developmental stages (Figs. 13-16). The granules are smaller and confined to the perinuclear area. Both radial and additional wall thickening react positively to the ascorbic acid.

The ascorbic acid which is feeble at the time of differentiation of the tapetum, shows rapid increase upto meiosis and gradually declines and gets lost with maturity of the cells.

Discussion

Histochemical investigations have aided to the understanding of the various physiological processes involved during the development of anther tissues (Heslop-Harrison, 1972; Mascarenhas, 1975). In *Psophocarpus tetragonolobus*, the sporogenous cells exhibit high content of RNA, protein, moderate ascorbic acid and feeble PAS positive substances. Lack of starch accumulation was noted in many plants investigated earlier (Panchaksharappa and Rudramuniyappa, 1974; Rudramuniyappa and Panchaksharappa, 1983). However, presence of PAS positive starch grains are reported in *Euphorbia* (Rudramuniyappa and Annigeri, 1985). The present study in *Psophocarpus* differs from *Kalanchoe* (Rudramuniyappa and Annigeri, 1984) and agrees with *Triticum* (Rudramuniyappa and Panchaksharappa, 1980) and *Parthenium* (Rudramuniyappa, 1984) in possessing ascorbic acid in the cytoplasm of the sporogenous cells. A reduction in RNA and protein are noted in meiocytes. This reduction is a general feature (Panchaksharappa and Rudramuniyappa, 1974; Rudramuniyappa and Annigeri, 1984). The spectacular decline of RNA in the meiocytes coincides with peak activities of acid phosphatase and RNAase. This was linked with the breakdown of ribosomes (Knox *et al.*, 1971). Ascorbic acid content in meiocytes of *Psophocarpus* is high agreeing with *Kalanchoe* (Rudramuniyappa and Annigeri, 1984). Deposition of a strongly staining additional PAS positive wall, around each meiocyte was noted in the present study. The present study supports earlier reports on PAS positive nature of callose thickening (Panchaksharappa and Rudramuniyappa, 1974; Rudramuniyappa and Annigeri, 1984, 1985). The callose thickening might act as a source of carbohydrates to the meiocytes during meiosis. The common occurrence of ascorbic acid in the cytoplasm and cell walls of the spore tetrads, strengthens the possibility of its involvement in the biosynthesis and/or mobilization of pollen wall materials. Soon after the release of the spores, RNA and protein declines slighty in the cytoplasm. Such reduction has been noticed in many other taxa (Heslop-Harrisson, 1972). Pollen grains store high RNA, protein, insoluble carbohydrates and ascorbic acid. The exine constitutes a complex of different materials and it reacts strongly with Azure-B giving a green colour in the present study and in *Kalanchoe* (Rudramuniyappa and Annigeri, 1984) and *Euphorbia* (Rudramuniyappa and Annigeri, 1985).

The accumulation of polysaccharides, RNA, proteins and ascorbic acid in *Psophocarpus*, in *Kalanchoe* (Rudramuniyappa and Annigeri, 1984) and various enzymes in *Helianthus* and *Lolium* (Vithange and Knox, 1980) and *Datura* (Hegde and Andrade, 1982) speaks in favour of storage and secretory nature of the tapetum.

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