CYTOCHEMISTRY OF ANTHER DEVELOPMENT IN *CICER ARIETINUM* L. AND *ARACHIS HYPOGEA* L.

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Abstract

The polysaccharides, proteins, ascorbic acid and RNA have been studied during the development of anther of *Cicer arietinum* L. and *Arachis hypogea* L. The cell-wall layers, archesporium and primary sporogenous tissue are PAS positive. The tetrads of microspores also show increased amounts of polysaccharides, proteins and ascorbic acid. The secretory tapetum is rich in polysaccharides, proteins and RNA. The cytoplasm of mature pollen grains has high level of polysaccharides, proteins and RNA.

Introduction

Biochemical constituents of the tissue in any heterogeneous structure can be assessed by histochemical methods. The aim has been to follow changes during the development of the sporogenous tissues, spores and the associated tepetum in certain cell constituents in tissues, namely polysaccharides, proteins, ascorbic acid and RNA.

Material and methods

The plants of *Cicer arietinum* and *Arauchis hypogea* were grown in the field of the college of Agriculture, Nagpur. Polysaccharides, proteins, ascorbic acid have been localised during the development of anther.

Flower buds before and after anthesis were fixed in Cornoy's fluid for the localization of polysaccharides, proteins and RNA for 24 hours and immediately washed with 70 % ethanol The flower buds were also fixed in acidified silver nitrate solution (5 gm of silver nitrate in 66% of ethyl alcohol) for 5 to 7 days for locatization of ascorbic acid, and washed with a solution of 45 ml of 70% ethyl alcohol and 5 ml of ammonia for two to three times. The flower buds were dehydrated in alcohol-n-butanol series, and embedded in paraffin. The blocks were sectioned on a rotary microtome at 6-8 μm thickness. Serial sections were mounted on clean slides smeared with Mayer's albumin, and these slides were then processed for the localization of polysaccharides, proteins, ascorbic acid and RNA. The following histochemical techniques were employed:—

1. Polysaccharides--Periodic--acid Schiff's reaction (Jensen, 1960).
2. Proteins-Ninhydrin-schiff's reaction (Yasuma & Ichikawa, 1953).
3. RNA-Azure B method (Flax & Himes, 1950).
4. Ascorbicacid-Silver nitrate method (Dave *et al.*, 1968).

Observations

Polysaccharides-young anther primordium of *C. arietinum* and *A. hypogea* shows poor polysaccharides contents (Pl. 1, fig. 1). The sporogenous cells, pollen mother cells, micropore tetrads and pollen following their differentiation show increasing PAS positive storage grains cytoplasm. The cell walls of the sporogenous cell and PMC's are distinct and react strongly to PAS test (Pl. 1, fig. 2). At the time of initiation of meiosis, accumulation of additional PAS positive material around the PMC's is observed. In both *C. arietinum* and *A. hypogea*, cytoplasm of dyad and tetrad contain abundance of storage PAS postive grains. The tapetum from the beginning of its differentiation shows PAS positive storage. Tapetum is secretary type and shows low PAS positive stain during pollen maturation. The cytoplasm of the mature pollen is engorged with PAS positive starch grains (Pl. 1, fig. 3). The storage grains

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continue to be present in the middle wall layers, endothecium and epidermis. The endothecium thickening in *A. hypogea* also shows PAS positiveness.

Proteins—The anther at early sporogenous stage contains less amount of proteins in *C. arietinum* and *A. hypogea*. As the tissues grow, there is gradual accumulation of proteins. This is invariably evident when the tissue has been transferred into PMC's. High amount of proteins is maintained until the prophase I of meiosis. The nuclear proteins in general are higher than the cytoplasmic proteins. However, the early microspores show decrease in the protein contents. When the microspores are separated from the tetrads, a gradual increase of proteins takes place until they mature (Pl. 1, fig. 4). The microspores within the tetrads possess protein positive wall. The exine of the pollen also reacts with proteins. The tapetum consistantly possesses higher quantity of proteins, until the tissue is obliterated (Pl. 1, fig 5). Other tissues, such as, anther wall layer and connective show poor protein contents. Protein contents increase further during pollen separation.

Ascorbic acid—In the young primordium AA reduced silver grains are sparsely distributed. At the time of differentiation of sporogenous tissue AA content increases. At times the PMC's show small grains in the cytoplasm. The tapetum exhibits low AA content in the beginning but the quantity increases as it develops further, and thus at meiosis a high content has been noticed (Pl. 1, fig. 6). In the developing microspores AA begins to deposit in the form of silver grains on their wall. The mature pollen do not show AA content in cytoplasm.

RNA—Undifferentiated anther show low content of RNA. As the differentiation takes place, the archesporium also shows bright green stain indicating high RNA content The tapetal cells, dyad and tetrad have a rich RNA content in the nucleus as well as in the cytoplasm. The microspores show gradual increase in RNA (Pl. 1, fig. 7) after they are released from the tetrad. The tapetum consistantly possesses higher quantities of RNA until the tissue is obliterated. The anther wall layers and connective contain low RNA. The degenerated layer between the endothecium and tapetum is RNA positive. Mature pollens also show rich RNA content.

Discussion

Each tissue of the microsporangium in the anther differs both in structure and constitution and hence in function. The soluble carbohydrates has been variously reported as one of the ATP source in the living tissue for the metabolic activities. Further it is one of the essential ingradients in cell wall formation. Low polysaccharides content in archesporium and sporogenous tissue has also been reported (Heslop-Harrison, 1972; Panchaksharappa & Rudramuniyappa, 1975). Similarly PAS +ve granules have been found in *Euphorbia* (Rudramuniyappa & Annigeri, 1985). The cytoplasm of pollen mother cell is highly PAS positive in *C. arietinum* and *A. hypogea*.

The meiocytes during meiosis undergo profound changes as revealed through EM and cyto/histochemical work (Heslop-Harrison, 1972; Bhandari, 1984). In a few cases, accumulation of PAS+ve polysaccharide material was observed around the meiocytes and the tetrads (Bhandari & Sharma, 1983). A few visible changes have been seen during differentiation of organelles in the meioytes Reduction in the amount of RNA, isolation and insulation of the individual meiocytes and microspores by a callose wall (Knox *et al.*, 1971; Mascarenhas, 1975; Bhandari, 1984).

The protein synthesis is prominent in the premeiotic stage of *Rheo* and *Paeonia* (Albertini, 1967; Sauter, 1968). Sporogenous tissue shows increase in its cytoplasmic and nuclear proteins and RNA during its development in *A. hypogea* and *C. arietinum*. The wall layers of the cells of the sporogenous tissue also show rich PAS positive stain PMC are rich in proteins and RNA. Successive increase in proteins and RNA appear to be of primary importance for tissue growth and further differentiation. It is known that ascorbic acid activates nucleic acids and protein synthesis in the cells (Chinoy, 1962).

AA is one of the important physiologically active ingradients and has been known to enhance the synthesis of nucleic acids (Chinoy *et al.*, 1971). Pollen wall morphogenesis is complex process, involving synthesis, degradation, mobilization and transfer of precursor materials from the tapetum to pollen wall. In the anther, the major supply of metabolites is through the vascular tissues. Connective and wall layers in it

Table 1—Comparative pattern of localization of polysaccharides, proteins, ascorbic acid and RNA in ths successive stages of anther development.

Name of the plant	Biochemicals	*Pre-Meiotic* Early sporogenous	*Pre-Meiotic* Late sporogenous tapetum	*Meiotic* Meiosis I tapetum	*Meiotic* Miosis II tapetum	*Post meiotic* Micropores tapetum	*Post meiotic* Shedding pollen tapetum	Remarks
1. *Cicer arietinum*	Polysaccharides	—	+	++	+++	+++	+++	Polysaccharide localization in both the plants is same in sporogenous tissue and increasing cytoplasmic tinge in spores and tapetum during successive stages of anther development.
2. *Arachis hypogea*	—do—	—	+	++	++	+++	+++	
C. arietinum	Proteins	+	+	++	++	+++	+++	Protein localization is low in sporogenous tissue and increasing cytoplasmic tinge in spores and tapetum during successive stages of anther development.
A. hypogea	—do—	+	+	+++	++	++	+++	
C. arietinum	Ascorbic acid.	—	+	+	++	++	+	Localization of AA is low in sporogenous tissues while rich in spores. Tapetum also show-low localization of AA.
A. hypogea	—do—	+	+	++	++	++	++	
C. arietinum	RNA	+	++	++	+++	+++	+++	RNA localization is low in sporogenous tissue and increasing cytoplasmic tinge in spores and tapetum during its development.
A. hypogea	—do—	+	+	++	++	++	+++	

— Absent, + low, ++ medium, +++ high.

function as storage centres. Tapetum in all probability takes part in transferring the stored materials from anther wall layers into meiocytes and/or into the anther locule As is normally known the AA could also be synthesized from soluble carbohydrates and is probably deposited in the pollen wall as observed in the present study. So there is mutual interaction and utilization of biochemical substance which occur between sporophytic and gametophytic tissues within the anther during pollen formation and differentiation.

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Explanation of plate

Anther section of *C. arietinum* and *A. hypogea* tested for polysaccharides, proteins, ascorbic acid and RNA.

Plate 1

1-3. Anther section tested for Polysaccharides.
1. Sporogenous tissue shows low Polysaccharides in *C. arietinum*.
2. PMC's at prophase show rich Polysaccharides in *A. hypogea*.
3. Young and mature pollen. Note gradual increase in Polysaccharide content in *C. arietinum*.

4-5. Anther section tested for proteins.
4. Single-celled pollen. Tapetum retains high protein content in *C. aritinum*.
5. Note gradual increase of protein content in mature pollen. Wall layers are also rich in proteins in *C. arielinum*.
6. Rich deposition of AA in tapetum and microspores in *C. arietinum*.
7. A portion of mature anther wall layer showing tapering epidermis. Endothecial thickening react green with azure B Note mature pollen showing rich RNA in *A. hypogea*.