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HISTOCHEMICAL STUDIES ON ONTOGENY OF ANTHER WITH SPECIAL REFERENCE TO TOTAL PROTEINS IN *CLITORIA TERNATEA* LINN.

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ABSTRACT

The present histochemical studies were carried out to know the ontogeny of anther with special reference to total proteins, in *Clitoria ternatea* Linn. The anthers were bicelled and tetrasporangiate following dicotyledonous type of wall development. The wall layers comprised an epidermis, an endothecium, middle layers and a secretary tapetum. The microspore tetrads were tetrahedral and pollen grains were triporate. Realizing the importance, the species was undertaken for histochemical studies with reference to total proteins during ontogeny of the anther and anthesis in *C. ternatea* Linn.

Figures : 08 References : 11 Table : 00

KEY WORDS : Anthers**,** *Clitoria ternatea* Linn., Histochemistry, Total proteins.

Introduction

Clitoria ternatea Linn. belongs to family Fabaceae (Leguminosae). It is one of the ornamental climber plants, cultivated in India and Northern Australia due to having forage and some medicinal values. It could replace *Medicago sativa*[2]. Realizing importance, the study was undertaken for histochemical studies with special reference to total proteins during ontogeny of the anther and anthesis in *Clitoria ternate*. The growth and differentiation are vital processes in the development of plant organism. It starts from the embryonic apices. It involves both vegetative and reproductive growth phases in the plant organization. The flower, an organ of reproduction, shows many functions during its development, ultimately to produce renewed individuals with good heredity changes for survival. Each organism has its own characteristic in sexual reproduction being marked by variations within a series of different co-ordinated steps, such as, differentiation of sporogenous cells, meiosis, cell isolation and insulation by a callose wall, attraction and recognition, cell fusion and the resting period. The origin of the anther and ovules from the reproductive shoot is basically comparable and similar, especially in their functions. Both micro- and megagametophytes determine very specific individuality of the sexual reproduction of each distinct species.

The entire plant organism is diploid and heterogeneous. Plant organs made up of tissues which are histologically different, showing cytological diversities. Anatomical and cytological diversities reflect biochemical and physiological variations. Morphological differentiations of plant organs are equally dependent on growth, differentiation, regeneration and biochemical correlation. Histochemical techniques locate the site of a particular reaction, thereby indicating change in the metabolism at a cellular level. It helps to understand the histochemical composition of the tissues concerned, their origin and differentiation[4].

Histochemical methods enable identification and localization of biochemical metabolites specific for the nature of cell wall,

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cytoplasm and nucleus, within the cells and tissues.

Biochemical constitution of a tissue in any heterogeneous structure can be assessed by standard histochemical methods. Histochemical methods applied on the tissue sections of fixed as well as fresh material alone reveal not only the biochemical content of each tissue, but also the physiological basis of their differentiation too.

Microsporogenesis is a serial but complex, ontogenetic process involves differentiation of archesporium and successive delimitation of different tissues from its specific to their functional areas, culminating in formation of the pollens, from pollen mother cells, after meiosis. The anther tissues are epidermis, endothecium, middle wall layers, tapetum and sporogenous tissue are quite different in their composition during growth and differentiation.

The main aim behind the present study on *Clitoria ternatea*. was to know the developmental organization of an anther and the functional role of their different tissues; and to explain the changes in the concentration of some metabolic macromolecular substances particularly total proteins during the successive growth and differentiation of anther using standard histochemical techniques under the light microscope.

Materials and Methods

The different developmental stages of flower buds and opened flowers of *Clitoria ternatea*. were collected and were fixed in Carnoy's fluid for 12 hrs. These collected floral buds were dehydrated, infiltrated and embedded in paraffin wax (52 to 54^0 C temp.). Then 8µm thick sections of floral buds and anthers from processed opened flowers were cut with rotary microtome and processed for the staining. The localization of total proteins was done by using mercuric bromophenol blue dye[4,6]. The results were recorded for development of anthers and localization of total proteins in the form of microphotographs. The total proteins stain blue or deep blue with stain and are expressed as rich or feeble or low to denote visual intensities of stain in the observations.

Observations

Epidermis: In the young anther, epidermis is rich in total proteins, both cytoplasm and nucleoli react strongly (Fig. 1). But as it matures the protein content declines (Figs. 4-6) and remains low until the layer degenerates (Figs. 7-8).

Endothecium: During early stages of anther development, endothecium is rich in total proteins similar to epidermis. During further growth as it looses RNA content it shows low quantity of proteins (Fig. 6). The fibrous thickenings are not uniform but it develops more fibrous thickenings towards connective. The endothecial thickenings at pollen stage show feeble response to protein test (Fig. 8).

Middle wall layers: It shows positive protein test when young, while during its growth proteins are much reduced in cytoplasm from sporogenous tissue to microspore stage but the nuclear proteins remain rich in them (Fig. 3, 5). At maturity these nuclear proteins reduce to low level (Fig. 6).

Tapetum: At the beginning the tapetum shows low protein content than the central sporogenous tissue (Fig. 1). The tissue when it is young not much vacuolated, but subsequently an increase in vacuolation is observed in it (Figs. 3,5,6). The tapetal cells towards the connective are more vacuolated than the cells present at parietal side and their size is also large. The quantity of protein increases as the sporogenous tissue prepares itself for meiosis and persists during the entire period of meiosis, but at pollen mother cell stage it declines. The cells at maturity become disorganized and appear separated, show decline in protein content (Fig. 6). Parallel increase in vacuolation is also noticed. At microspore stage tapetum starts degenerating and its content falls and finally lost completely (Fig. 7).

Sporogenous tissue: It shows insignificantly rich protein stainability. The nuclear proteins are always higher than that of the cytoplasm (Figs. 1-3). During formation of pollen mother cells, however, the concentration of proteins lowers down (Fig. 6).

Pollen grains: The cytoplasm of the released microspores shows gradual increase in the pollen contents, indicated by dark stainability. Additional deposited layer around the tetrad shows faint staining with protein test. Protein content in tetrads also declines (lowers) but it increases in the mature pollen grain (Fig. 7). The anthesis *i. e.* pollen grains dehisced at 2-celled stage. The exine shows no protein reaction while intine gives rich deep blue colour, indicating accumulation of rich protein (Fig. 8).

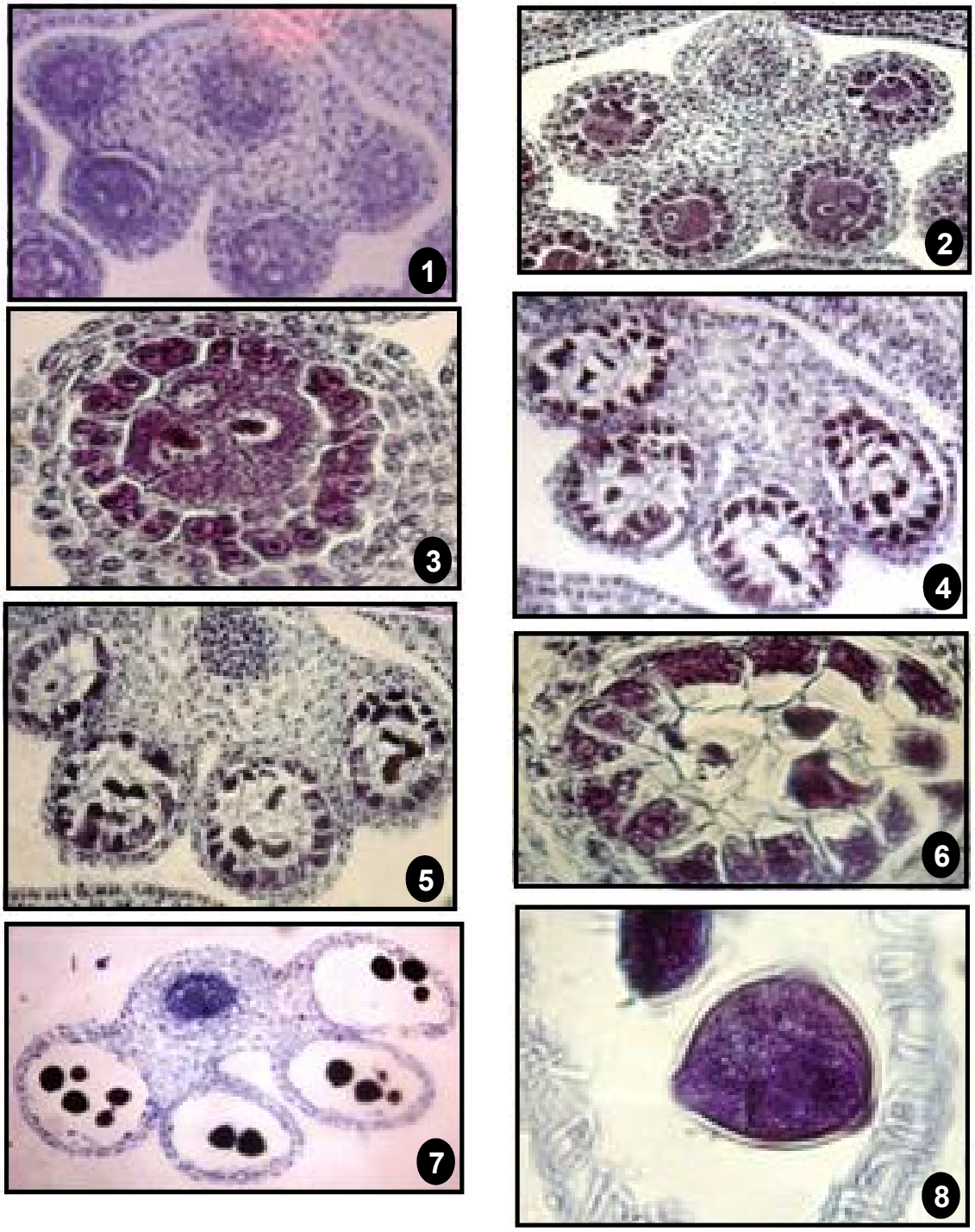

Plate I. Transections to know anther development in *Clitoria ternatea* Linn. for localization of total proteins showing epidermis, endothecium, middle layers, tapetum, sporogenous tissue and pollen grains having variations in blue colour as deep or feeble. (Original figures: 1, 4, 7 x84; 2, 5 x87 & 3, 6, 8 x300).

Results and Discussion

Histochemical assessment using light microscope is of specific advantage, in getting large quantity of data regarding biochemical basis of tissue and organ differentiation such as anther. The cell or the tissue differentiation in them depends on the synthesis and concentration of various biochemical metabolites. Histochemical studies have revealed that major biochemical substances like carbohydrates, proteins, lipids, enzymes, nucleic acids *etc.* play an important role in the development and differentiation of various structures in the anther[1,3-5,7-11]. Protein content is high in young anther epidermis but as it matures the protein content declines, which coincides with relative rise in protein content in endothecial layer. The two middle wall layers show identity with endothecium in its content in early stages. Degeneration of wall layers relate with rise in metabolic content in tapetum. The tapetum is single layered, secretary and nutritive in nature. The tapetum, until meiosis is completed, stores very high protein content, both in the cytoplasm and nuclei. The tapetum being highly vesiculated and in all probability it functions as a storage tissue in the anther. During meiosis and separation of microspores, increase in proteins is very unique. The mature pollen grain exhibit rich protein content. In the sporogenous tissue, pollen mother cells and microspore tetrads, the total protein content is high. This might be the prerequisite for pollen tube growth. The interaction and probable role of the protein content in the anther tissues is discussed by making use of relevant previous data.

The present study dealt with growth differentiation and role of total proteins during ontogeny of anther in *C. ternatea* L. An increase and decrease in concentrations of the total proteins depend on the activity of the tissues involved during pollen grain formation.

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