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HISTOCHEMICAL AND CYTOPHOTOMETRIC STUDIES ON SHOOT APEX OF *TABERNAEMONTANA DIVARICATA* (LINN) R. Br.

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Histochemical and Cytophotometric quantification in different developmental stages of shoot apex of *Tabernaemontana divaricata* showed distribution pattern of DNA, RNA, total proteins and insoluble polysaccharides and a cytohistological zonation pattern superimposed upon a tunica-corpus organisation. DNA content per nucleus and RNA and total protein content per cell were higher in the peripheral zone and lower in the central mother cell zone of the vegetative apex. The transitional and reproductive apices showed more metabolites in mantle layers as compared to the core. The entire apex seems to be involved in the reorganisation during transition to flowering.

Keywords: Cytophotometric quantification; Histochemistry; Shoot apex; *Tabernaemontana divaricata;* Zonation.

Introduction

Histochemical studies on shoot apices elucidate the changes in the apex prior to and during transition to flowering [1,2]. The present study deals with histochemical and cytophotometric quantification of different metabolites in the various zones of the apex at different developmental stages.

Materials and Methods

Histochemical tests were performed on apices of *Tabernaemontana divaricata* (Linn.) R. Br. in three ontogenetic stages viz., vegetative, transitional and reproductive. Apices were fixed in F.A.A. and Carnoy's fluid in the second week of every month throughout the year, and preserved in 70% alcohol. Materials were dehydrated, embedded in paraffin and serial longitudinal sections were cut at 7 µm. The staining procedures followed were : (a) DNA - Feulgen method[3] with perchloric acid as control; (b) RNA-Pyroninγ as a stain[4] with perchloric acid as control[5] (c) Total proteins-Mercuric bromophenol blue method[6] with acetylation[7] serving as control and (d) Insoluble poly-saccharides-Periodic acid-Schiff's reaction[8,9] with untreated material serving as control.

Cytophotometric quantification of DNA, RNA and total proteins was done by adopting the formula—Content per Cell or nuclear volume = Extinction value x Cell area or nuclear volume[10]. Cell area and nuclear area were determined from Camera lucida drawings of median longitudinal sections. Measurements of five cells were taken from each zone of the apex and the average worked out.

Observations

The distribution pattern of DNA, RNA and total proteins showed cytohistological zonation in vegetative and reproductive apices, The content/nuclear area for DNA and content/cell for RNA and total proteins were quantified cytophotometrically.

(A) *DNA:* Vegetative apices stained with Schiff's reagent for localisation of DNA showed distinct zonation with densely stained nuclei in the peripheral zone (PZ) and lighter stained nuclei in the axial tunica cells and central mother cell zone (CMZ) (Fig. 1). The reproductive apex showed a mantle-core organisation. Mantle cells showing darkly stained nuclei as compared to the nuclei of the core cells (Fig. 2). The nuclei are darkly stained at the loci where the initiation of floral whorls take place.

Cytophotometric quantification

shows the highest value for DNA content/ nucleus in the tunica and lowest in the CMZ in the vegetative apex. The PZ shows a value between these two (Fig. 7). In transitional apex the value is more in the mantle than the core and in the floral apex, it is marginally higher in the floral primordia as compared to the mantle and much lower in the core (Fig.7).

RNA: The axial tunica cells and CMZ cells are less pyroninophilic as compared to the PZ (Fig. 3). Deeper nuclear and cytoplasmic staining is evident at the loci for leaf initiation. The reproductive apex shows more nuclear and cytoplasmic RNA as compared to the vegetative apex the mantle layers being more pyroninophillic than the core (Fig. 4). The loci where floral primordia initiated showed darker stain. In the vegetative apex, RNA content/cell is highest in the PZ and lowest in the CMZ, the tunica showing slightly lower value than the PZ. In the transitional apex the value is higher in the mantle than the core whereas in the floret apex, there is marginal difference in mantle, core and floral-primordium (Fig. 7).

Total proteins: The distribution pattern of total proteins shows lightly stained axial tunica cells and CMZ and darkly stained PZ in the vegetative shoot apex (Fig. 5). In the transitional and reproductive apices mantle layers show more of total proteins as compared to the core. The floret apex shows almost uniform staining in the mantle and core except at the loci where the initiation of floral primordia takes place. The protein content/ cell follows a pattern identical to that of RNA (Fig. 7).

Insoluble polysaccharides: Vegetative apices stained with PAS reagent show darkly stained cell walls in the CMZ (Fig. 6). The external walls of the first tunica layer and the epidermis of the developing leaves take on a darker stain. The differentiating pith cells are also darkly stained. The cells of the mantle and core in the reproductive and floret apices are uniformly stained.

Discussion

The PZ, comparable to the anneau initial of the French School, exhibits a higher metabolic activity than the CMZ in the vegetative apex in terms of DNA, RNA and total protein content. This pattern of distribution of metabolites in the vegetative apex is in general agreement with previous reports[1,2,11,12]. The Anglo-Saxon School[13] believes that the centrally located region of the apex (equivalent to the meristeme d' attente of the French School) does divide and contribute cells to PZ. The mitotic figures in some of the cells of CMZ have also been observed in *Tabernaemontana divaricata* shoot apex.

Regarding the reproductive apex, the present study agrees with the interpretation given by Gifford[11] and others[12] that all zones became active and the entire apex is involved in the formation of the reproductive apex. There is a shift in plane of divisions and direction of enlargement of many distal cells of the CMZ of the vegetative apex so as to form the mantle layers together with the tunica and PZ. The mitotic activity in the mantle layers is largely responsible for floret and floral primordia formation while core forms the inner bulk.

Cytophotometric quantification of nucleic acids and total proteins in the vegetative apex reveals a consistently higher RNA content/cell in the PZ and lowest in the CMZ. Total protein content/cell also follows more or less similar lines. It is significant that the CMZ shows lower values for DNA content/ nucleus and higher values for PZ indicating greater mitotic activity in this zone. The increase in metabolites in the mantle layers of the reproductive apex is evident as this is the site of floral primordia formation. A lower concentration of RNA in the axial cells as compared to the peripheral cells of *Datura* reproductive apex was reported[14], whereas

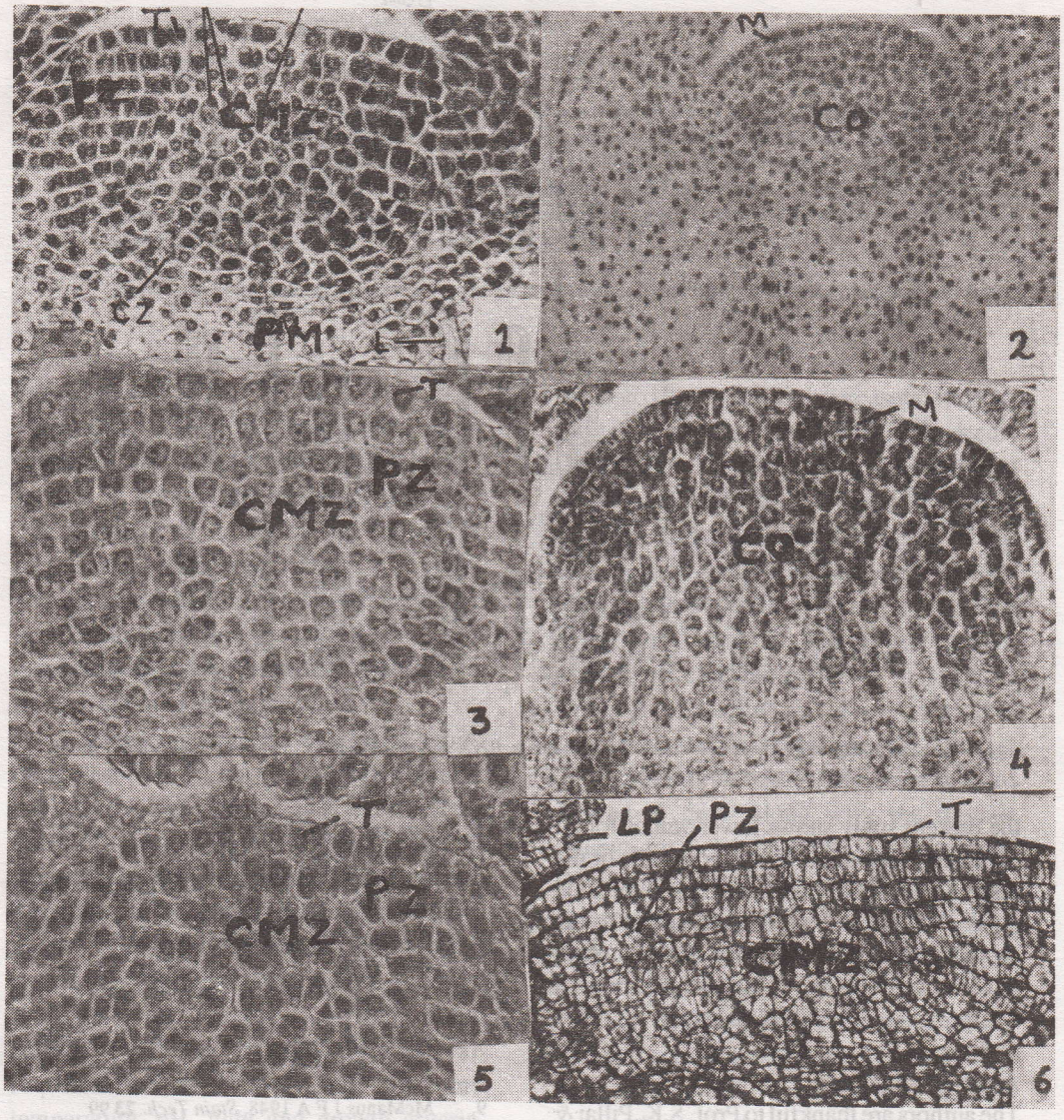

Fig. 1- 6; Median longitudinal sections of the shoot apices; 1, Vegetative shoot apex stained for DNA (X 400); 2, Reproductive apex stained for DNA (X400); 3, Vegetative shoot apex stained for RNA (X400); 4, Reproductive apex stained for RNA (X500); 5, Vegetative shoot apex stained for total proteins (X500); 6, Vegetative shoot apex stained for insoluble polysaccharides (X500); CMZ, Central mother cell zone; CO, Core; LP, leaf primordia; PM, pith meristem; PZ, peripheral zone; M, mantle; T, tunica.

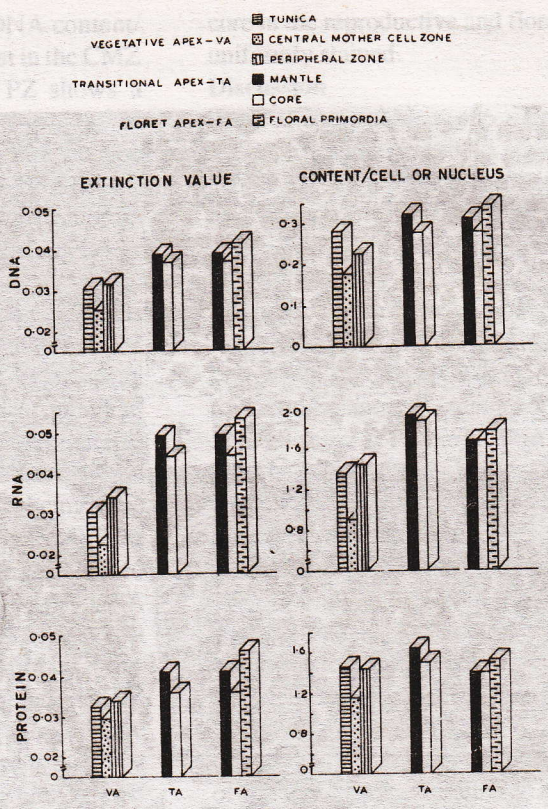

Fig. 7: Histogram depicting Cytophotometeric data of DNA, RNA and total proteins in various zones of the shoot apex at different developmental stages.

the present study shows lower values for all the three metabolites in the axial cells of the reproductive apex. Changes in apical zonation during transition from the vegetative to the reproductive phase reported in this study agrees with more commonly accepted interpretation that the entire apex is involved in the transformation[11,14].

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