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MORPHO-HISTOCHEMICAL STUDIES OF THE SHOOT APEX OF *NIGELLA SATIVA* L.

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Abstract

The shoot apical meristem of *Nigella sativa* L. was studied from the mature embryo to flowering. The anatomical study was followed by histochemical localization and cytophotometric quantification of DNA, RNA and total proteins. The plumular apex showed a tunica corpus organisation. The first evidence of zonation occurred in the shoot apex of the three-day-old seedling apex. Zonation was well-established in the five day old seedling shoot apex which remained till the age of four weeks. It showed a single layered tunica covering a lightly stained central mother cell zone (CMZ)[1], a subjacent pith meristem (PM) and a densely stained peripheral zone (PZ). The reproductive apex showed a mantle core organisation. The present data agreed with the interpretation that all zones become active and the entire apex is involved in the transformation.

Key words: *Nigella sativa;* shoot apex; zonation; histochemical localization; cytophotometric quantification

Introduction

Growth and differentiation involve physiological and biochemical changes, some of which are histochemically detectable. Over the past few decades there have been increasing attempts to study changes in the relative distribution of metabolites in the apical meristem and to correlate the data with the anatomical and histological changes (Gifford and Tepper 1961, 1962a, b; Nougarède *et al.*, 1965; West and Gunckel 1968a, b; Corson and Gifford, 1969; Molder and Owens, 1972; Kavathekar and Pillai, 1979; Goyal *et al.*, 1982, 1983).

The present study describes the inception, development and ontogenetic variations in zonation with reference to histochemical localization and cytophotometric

[1] Abbreviations: C—corpus; CMZ—central mother cell zone; CO—core; CY—cotyledons; FP—floral primordium; LP—leaf primordium; M—mantle; PM—pith meristem; PZ—peripheral zone; T—tunica.

quantification of DNA, RNA and total proteins at the shoot apex of *Nigella sativa* L. from the plumular to flowering stages.

Materials and Methods

Seeds of *Nigella sativa* L. were germinated in petriplates lined with moist filter paper. Shoot apices were fixed at 24 hour intervals for the first seven days after seed wetting and then at weekly intervals till flowering from seedlings raised in pots. Materials were fixed in FAA (for anatomy and total protein localization) and Carn'B (for localization of nucleic acids) processed through TBA series, embedded in paraffin wax and sectioned at 7 μm. For anatomical studies slides were stained with safranin and light green with combination of tannic acid and ferric chloride (Johansen, 1940) were used for anatomical studies. The staining procedures used for various cellular components are as follows:

(a) DNA—Feulgen technique (Feulgen and Rosenbeck, 1924) with perchloric acid serving as control (Erickson *et al.*, 1949).

(b) RNA—Pyronin Y as a stain (Tepper and Gifford, 1962) with perchloric acid as control.

(c) Total proteins—Mercuric bromophenol blue method (Mazia *et al.*, 1953) with acetylation (Jensen, 1962) serving as control.

A cytophotometer was used to measure the intensity of the stain from histochemical preparations following the absorption laws of Lambert and Beer (Shah *et al.*, 1975). Two parameters *viz.*, extinction value and content per cell or nucleus were calculated from absorption values conveying metabolic density in a particular area of the cell by these formulae:

$$\text{Extinction value} = \log I_0 - \log I_s$$

I_0 = Light passing through a blank portion of the slide.

I_s = Light passing through histochemical preparation (Stained area).

$$\text{Content per cell/nucleus} = \text{Extinction value} \times \text{Cell area or nuclear area}$$

These values were measured in arbitrary units (AU) at cells of each zone of the apex.

Results

Anatomy

The Plumular Apex: The plumular apex (apex of the mature embryo) was slight concave, 40.0 μm wide and flanked by the cotyledons. It showed a single layered tunica covering a homogeneous corpus. The cells of the subjacent corpus were irregularly arranged and often more vacuolated (Fig. 1).

The Vegetative Apex: The non-zonate plumular apex became zonate during

growth. The inception of zonation is noted in the three-day-old seedling shoot apex as denser staining of cells on the flanks in comparison to the axially located cells of the corpus. The seedling shoot apex from the five-day-old seedling onwards showed a well-established cytohistological zonation with a lightly stained central mother cell zone (CMZ), a densely stained peripheral zone (PZ) and a pith meristem (PM).

The apex was a low to high dome depending on the plastochronic stage. A gradual age-related increase in height and width of the apex was maintained from germination till five weeks. Height and width of the shoot apex varied from 30.0 μm to 120.4 μm and 90.2 μm to 200.0 μm, respactively. The vegetative apex had a single layered tunica with a few lightly stained axial cells. The axial CMZ immediately proximal to the tunica cells at the summit of the apical dome showed a group of lightly stained cells. The PZ formed a 2-3 cell layers around the CMZ and PM. The cells of the PZ were darkly stained and showed anticlinal and periclinal divisions resulting in regular cell files which broaden proximally and formed the site for initiation of lateral primordium (Fig. 2).

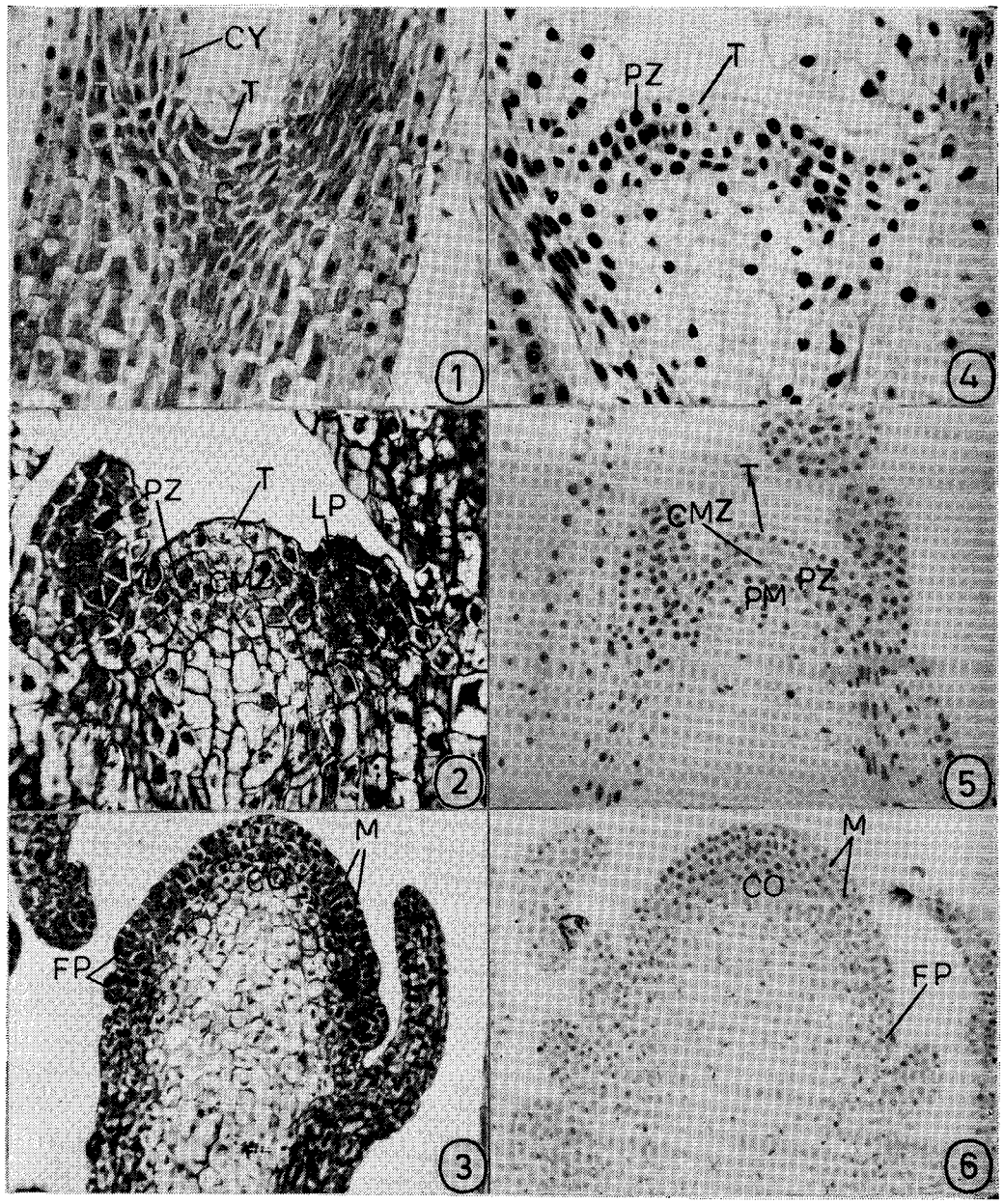
The Reproductive Apex: At 6-weeks the vegetative apex changed into the transitional apex having a height and width of 160.0 μm and 240.0 μm. Zonation decreased and there was an increased stratification in the corpus region leading to the establishment of a 3-4 layered mantle. The cells of the mantle layers were smaller, densely stained and more chromophilic as compared to the larger, vacuolated, lighter stained core cells. The floral apex also showed a mantle-core organization. The sepal, petal, stamen and carpel primordia arose successively in acropetal arrangement (Fig. 3). After producing a number of stamen primordia the whole floral apex was consumed in the formation of carpels.

Histochemistry and Cytophotometry

Distribution pattern and cytophotometric quantification of DNA, RNA and total proteins generally support the anatomical data.

DNA: In the plumular apex the tunica and corpus showed uniformly stained nuclei. The 3-day-old seedling apex showed darkly stained nuclei in the PZ as compared to the CMZ (Fig. 4). The vegetative apex showed clear zonation having darkly stained nuclei in the cells of the PZ as compared to the CMZ (Fig. 5). The zonation present in the vegetative apex disappeared during changeover to the reproductive phase. The nuclei of mantle cells were darkly stained as compared to core nuclei in the transitional and floral apices. Loci for floral primordium showed darker nuclei (Fig. 6).

The cytophotometric data indicate higher extinction value in the tunica of the plumular apex whereas the amount per nucleus was lesser in tunica as compared to the corpus nuclei. In the early zonate and vegetative apex the CMZ



Figs. 1-6. Median longitudinal sections of the shoot apical meristem stained from safranin and light green (1-3) and for DNA localisation (4-6).
Fig. 1. The plumular apex (×400).
Fig. 2. The vegetative apex (×500).
Fig. 3. The reproductive apex (×250).
Fig. 4. The 3-day old seedling apex (×400).
Fig. 5. The vegetative apex (×400).
Fig. 6. The reproductive apex (×250).

showed lesser values than the PZ and tunica. In reproductive apices, higher values were obtained by the mantle in comparison to the core. The nuclei of floral primordium showed highest values (Table 1).

RNA: In the plumular apex staining for RNA showed an almost uniform distribution pattern in the tunica and corpus cells. The seedling shoot apex and the vegetative apex showed clear cytohistochemical zonation, the CMZ was less pyroninophilic in comparison to the PZ and tunica (Fig. 7). In the transitional apex cytoplasmic staining for RNA was more intense in the mantle layers as compared to the core (Fig. 8). The loci for floral primordium showed darkly stained cells.

The extinction value was higher in the tunica in the plumular apex whereas content per cell was higher in the corpus. The seedling and vegetative shoot apices showed higher values in the PZ in comparison to the tunica and CMZ. In the reproductive apices the extinction value was highest in the floral primordium followed by the mantle and core. The content per cell showed highest value in the mantle followed by the floral primordium and core (Table 1).

Total Proteins: Staining from bromophenol blue showed a uniform distribution in the cells of the tunica and corpus of the plumular apex. The seedling and vegetative shoot apices showed lower intensity in the axial cells. The PZ showed darker staining (Fig. 9). In the reproductive apices the mantle layers were uniformly darkly stained in comparison to the core-cells. The loci for floral primordium showed comparatively darker staining (Fig. 10).

In the plumular apex the extinction value was higher in the tunica whereas content per cell was higher in the corpus. The CMZ showed lowest values in the seedling and vegetative apices. The content per cell was highest in the mantle region of the reproductive apices (Table 1).

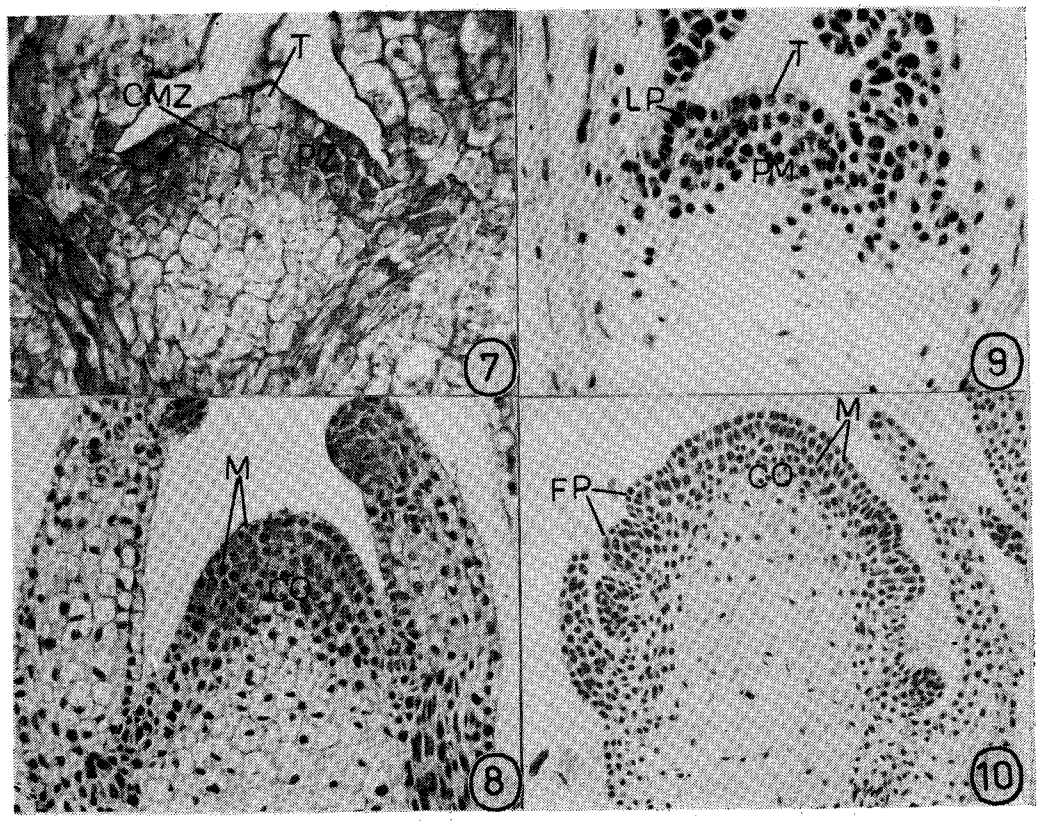
Discussion

Ontogenetic studies on the angiosperm shoot apices involving all developmental stages from germination to flowering are not many (Mauseth 1978a, b; Pillai *et al.*, 1984; Goyal and Pillai, 1985a, b). Data on shape and size presented in this study fail to establish any correlation between apex shape and age of the plant. A gradual age-related increase in size of the apical dome is followed by the maximum size of the reproductive apex.

The present study supports Mauseth's (1978a) contention that initiation of zonation in the seedling apex is not directly related to age or the number of plastochronic cycles. It is significant that the first zone to be established is the PZ (Goyal *et al.*, 1980; Pillai *et al.*, 1984) and following this, the CMZ cells show greater vacuolation and indications of low mitotic activity.

Table 1. *Cytophotometric data of DNA, RNA and total proteins in the different zones of the shoot apex (mean value±standard error)*

Stages	Zone	DNA		RNA		Total proteins	
		Extinction value ($\times 10^{-2}$)	Content/nucleus	Extinction value ($\times 10^{-2}$)	Content/cell	Extinction value ($\times 10^{-2}$)	Content/cell
Plumular apex	T	7.970±0.628	2.351±0.058	5.687±0.123	6.253±0.135	9.566±0.134	10.519±0.147
	C	7.840±0.212	2.405±0.065	5.318±0.201	7.028±0.265	9.154±0.219	12.111±0.289
Seedling apex	T	5.935±0.125	1.736±0.036	4.104±0.094	3.099±0.146	3.747±0.182	2.829±0.137
	CMZ	4.703±0.389	1.087±0.028	3.393±0.175	2.615±0.130	3.393±0.175	2.615±0.135
	PZ	6.935±0.125	1.898±0.034	4.342±0.158	3.414±0.124	4.227±0.257	3.324±0.201
Vegetative apex	T	4.104±0.194	1.162±0.055	3.985±0.198	2.557±0.127	4.834±0.302	3.102±0.194
	CMZ	3.159±0.155	0.819±0.041	3.393±0.175	2.081±0.107	4.584±0.180	2.812±0.110
	PZ	4.580±0.071	1.234±0.019	4.342±0.158	2.751±0.101	5.939±0.289	3.762±0.183
Transitional apex	M	4.580±0.745	1.377±0.022	4.584±0.180	3.029±0.119	7.046±0.084	4.655±0.055
	CO	3.682±0.158	1.072±0.046	3.628±0.158	2.465±0.107	5.689±0.222	3.866±0.151
Floral apex	M	4.580±0.074	1.473±0.023	4.342±0.152	4.150±0.151	6.435±0.208	6.150±0.199
	CO	3.628±0.158	1.101±0.048	3.747±0.182	3.404±0.165	5.564±0.164	5.055±0.149
	FP	4.703±0.123	1.513±0.039	4.584±0.180	3.470±0.136	6.810±0.166	5.156±0.126



Figs. 7-10. Median longitudinal sections of the shoot apical meristem stained for RNA (7-8) and for total proteins (9-10).
Fig. 7. The 4-day old seedling apex (×500).
Fig. 8. The transitional apex (×300).
Fig. 9. The vegetative apex (×400).
Fig. 10. The reproductive apex (×250).

The histochemical and cytophotometric data on zonation are in general agreement with previous reports (Corson and Gifford, 1969; Molder and Owens, 1972; Goyal *et al.*, 1983). Distribution pattern of DNA, RNA and total proteins follows the cytohistological zonation. In the plumular apex higher extinction values occur in the tunica for all the metabolites but the content per nucleus or cell is more in corpus, this can be attributed to the larger nuclei or cell size in the corpus. In the vegetative and reproductive apices maximum values are shown by the PZ and the mantle, respectively. This shows the higher activity of these regions in comparison to the other regions.

Recent controversy centers on the various interpretation of the functional aspects or roles of the different zones of the shoot apex during vegetative and reproductive phase. According to the French School of thought lateral organs in

the vegetative apex are produced by the activity of the subdistal and peripheral anneau initial. They also hold that the central zone which becomes mitotically active during transition to flowering is entirely responsible for the formation of bracts and inflorescence. Few investigators now agree with the latter generalization. The present data agree with more commonly accepted interpretation that all zones become active during transition and the entire apex is involved in the transformation.

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黑種草 (*Nigella sativa* L.) 莖頂形態—組織化學的研究

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本研究探討黑種草 (*Nigella sativa* L.) 自成熟胚至開花期植株之莖頂的分生組織。除做解剖研究外，並進行 DNA、RNA 及總蛋白質的組織化學定位和細胞光學定量之測定。胚芽頂端顯現外帶—內羣 (tunica corpus) 的組織分化。莖頂的帶狀分化 (zonation) 始於幼苗成長的第三天，至第五天帶狀分化已完全，並持續至第四週。帶狀分化包含單層的外帶包被染色很淺的中央母細胞區 (CMZ)、居下的髓分生組織 (PM) 及深染色的周圍區 (PZ)。生殖頂端顯現外套—軸心 (mantle core) 的組織分化。本試驗的數據支持「所有各區帶皆變成活躍，而整個頂端都與轉化 (transformation) 有關」的說法。