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Morpho-Histochemical Studies of the Shoot Apex of *Phlox drummondii* Hook.

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Structure and histochemical localisation of DNA, RNA and total proteins in the cells of the shoot apex of *Phlox drummondii* Hook. during its defferent developmental stages have been investigated. Their cytophotometric quantification was also undertaken. The plumular apex shows a tunica-corpus organisation. The first evidence of zonation occurs in the shoot apex of a 3-day old seedling. Zonation is well established on the fifth day after seed-wetting. The shoot apex shows a single layered tunica covering a lightly stained central mother cell zone, a subjacent pith meristem and a densely stained peripheral zone. The reproductive apex shows a mantle-core organisation. The peripheral zone in the vegetative apex and the mantle layers in the reproductive apices show higher concentration of metabolites.

Key Words: *Phlox drummondii*, **Shoot apex, Zonation**

Introduction

During the past few decades the shoot apical meristem has been investigated from different angles using different techniques and avenues of approach. Profound changes occur in the shoot apex during development from the embryonic to the reproductive phase. Only a few qualitative ontogenetic studies have been reported on angiosperms (Gifford & Tepper 1962a, b, Nougaréde 1965, Corson & Gifford 1969, Jacqmard et al. 1972, Orr 1978, Kavathekar & Pillai 1979).

The present study describes the inception, development and ontogenetic variations in zonation with reference to histochemical localisation and cytophotometric quantification in the shoot apex of *Phlox drummondii* Hook. from its plumular to the flowering stage.

Materials and Methods

Seeds of *Phlox drummondii* Hook. were germinated in petriplates lined with moist filter paper. Shoot apices were fixed at 24 hr 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 localisation) and Carnoys-B (for localisation

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-ferric chloride (Johansen 1943) were used for anatomical studies. The staining procedures used for various cellular components are as follows:

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

(*b*) RNA — Pyronin Y as a stain (Tepper & 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:
Extinction value=$Log\ I_0 - Log\ I_s$

where, I_0 is the light passing through a blank portion of the slide, and

I_s, the light passing through histochemical preparation (stained area)

Content per cell/nucleus=Extinction value × *Cell area or Nuclear area*

These values are measured in arbitrary units (AU) for cells of each zone of the apex (tunica, corpus, central mother cell zone, peripheral zone, mantle, core and floral primordia) at different developmental stages (plumular apex, early zonate apex, vegetative apex, transitional apex and floret apex).

Results

Anatomical Analysis

The plumular apex: The plumular apex (apex of the mature embryo) is a low and narrow dome with a height of 16.0 μm and width of 130.0 μm, flanked by the cotyledons. It shows a single layered tunica covering a homogeneous corpus. The cells of the tunica and corpus are more or less uniformly stained (figure 1).

The early zonate apex: The inception of zonation is noted in the three-day old seedling shoot apex as cells on the flanks are densely stained (figure 2). The seedling shoot apex from the five-day old seedling onwards shows 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). A single tunica layer covers the shoot apex.

The vegetative apex: The vegetative apex is a low or high dome depending on the plastochronic stage. A gradual age-related increase in size of the apex is maintained from germination till five weeks as the height increases from 10.2 μm to 45.4 μm and the diameter from 60.0 μm to 120.6 μm. The vegetative apex has a single layered tunica with a few lightly stained axial cells. The PZ forms a cylinder of 3-4 cell layers around the CMZ and PM. The cells are densely stained and show anticlinal and periclinal divisions (figure 3).

The reproductive apex: The inflorescence is a cyme. After five-weeks the vegetative apex changes into the broad transitional apex having a height and width of 72.0 μm and 172.2 μm respectively. Zonation decreases and there is an increased stratification of the surface layers. This results in a mantle-core organisation. The 3-4 mantle layers are more chromophilic and cover the lighter vacuolated cells of the core (figure 4).

The floret apex shows a mantle-core organisation with a 2-3 layered mantle of densely stained cells.

Histochemistry

Distribution patterns and cytophotometric quantification of DNA, RNA and total proteins generally support the concept of cytohistological zonation.

DNA: In the plumular apex the tunica and corpus show uniformly stained nuclei except for a few axially located cells (figure 5). In the early zonate apex the PZ has densely stained nuclei as compared to the CMZ. The nuclei of mantle layers are darkly stained as compared to core nuclei (figure 6). Loci for floral primordia show darker nuclei (figure 7).

RNA: The plumular apex shows a more or less uniform distribution pattern in tunica and the subjacent corpus cells. In the early zonate apex and the vegetative apex, the cells of the CMZ are less pyroninophilic as compared to the PZ (figures 8, 9). The axial tunica in the vegetative apex is marginally lighter than the peripheral tunica (figure 9). In the transitional apex RNA increases in all the zones and it shows a distribution pattern following the mantle-core organization. The loci for floral primordia are more pyroninophilic.

Total proteins: Distribution pattern of total proteins follows that of RNA. The plumular apex is uniformly darkly stained indicating storage of proteins in the embryo. The staining intensity decreases in the early zonate apex. The cells of the CMZ are lightly stained in both the early zonate and the vegetative apex (figures 10, 11). The reproductive apices show darker stained mantle layers, lighter core and deeply stained loci for floral primordia (figure 12).

Cytophotometry

Data on cytophotometric quantification of DNA, RNA and total proteins in the different zones of the shoot apex at various developmental stages are given in table 1.

Discussion

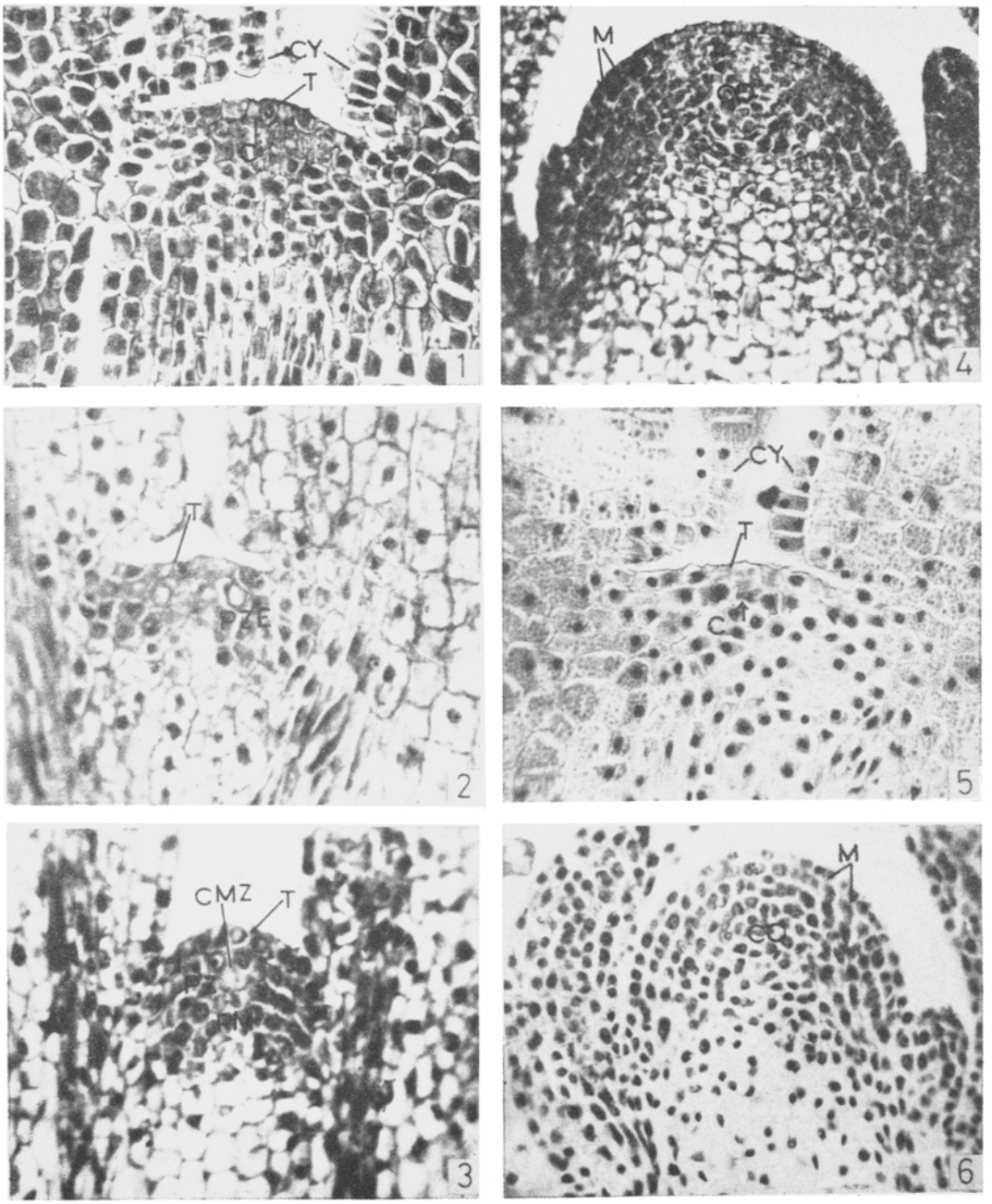
The present study shows an age related increase in size of the shoot apex as has been reported by others (Rogan & Smith 1974, Mauseth 1978a,b, Goyal et al 1980).

There are few reports which trace the origin, development and establishment of zonation in the angiosperm shoot apex. The data presented here support Mauseth's (1978a) conclusion that initiation of zonation in the seedling shoot apex is not directly related to shape, size or the number of plastochronic cycles and that the PZ is the first zone to get established.

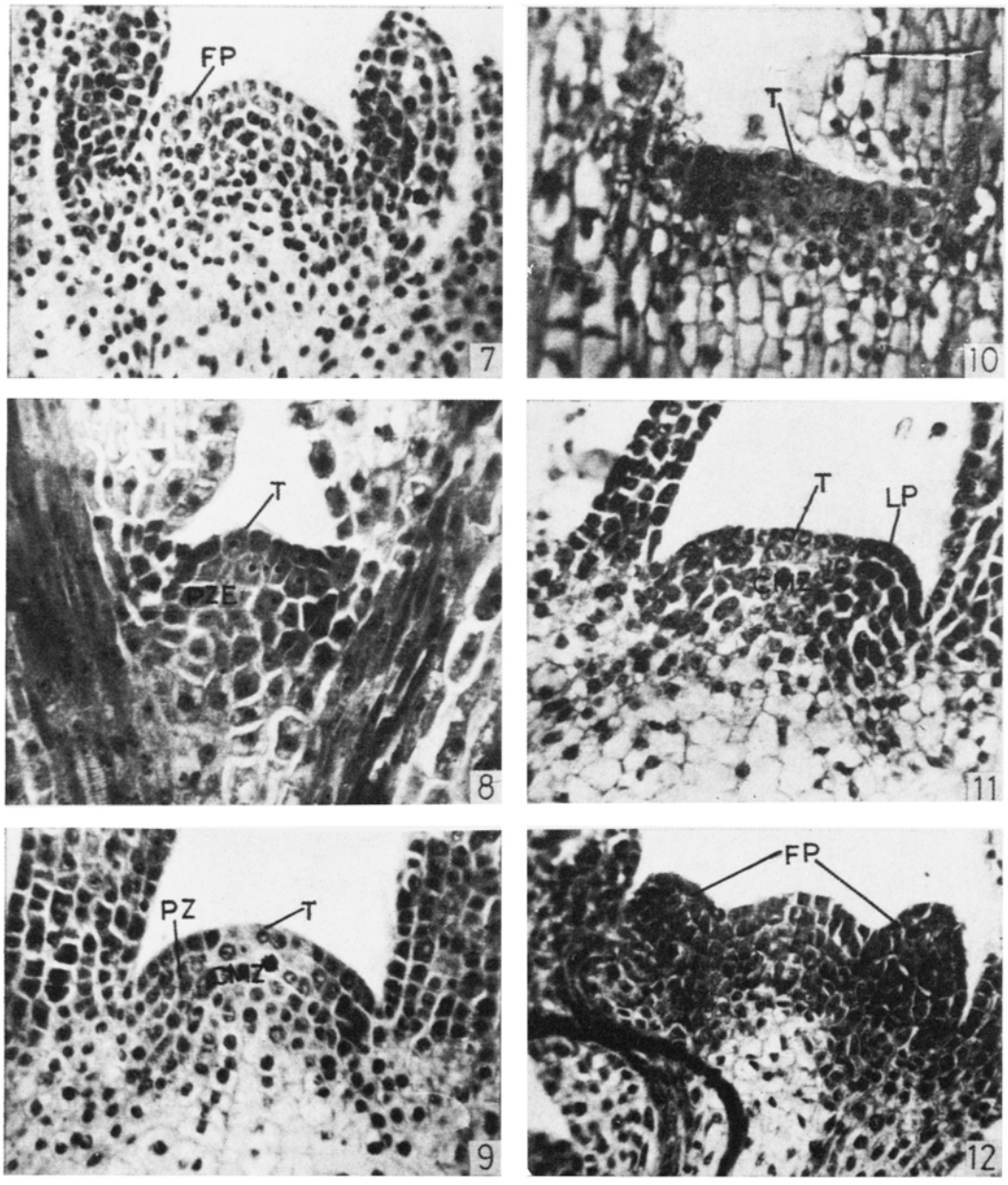
The results of histochemical localisation and cytophotometric quantification of DNA, RNA and total proteins follow a distribution pattern closely resembling the cytohistological zonation. These are in general agreement with previous reports (Corson & Gifford 1969, Molder & Owens 1972, Kavathekar & Pillai 1979, Goyal et al. 1983).

The downward trend showed by total proteins from the plumular to the early zonate apex can be attributed to the utilisation of storage proteins during germination and growth. The difference between values for RNA content and total proteins is very little in the vegetative and reproductive apices as compared to that in the plumular apex. This can be attributed to the higher metabolic activity in the vegetative and reproductive apices as compared to the plumular apex.

Recent controversy centers on the various interpretations of the functional aspects of the different zones of the shoot apex during vegetative and reproductive phases. There is an apparent parallelism in the zonate distribution pattern of DNA, RNA and total proteins reported in the vegetative apex and the views of the French School. The relative roles of the CMZ and PZ at transition



Figures 1–6, Median longitudinal sections of the shoot apexs tained from safranin and light green (1–4) and for DNA (5–6); **1,** The plumular apex (×400); **2,** The early Zonate apex (×500); **3,** The vegetative apex (×500); **4,** The transitional apex (×400); **5,** The plumular apex (×400); **6,** The transitional apex (×400)



Figures 7–12, Median longitudinal sections of the shoot apex stained for DNA (**7**), RNA (**8–9**) and total proteins (**10–12**); **7,** The florat apex (×400); **8,** The early zonate apex (×400); **9,** The vegetative apex (×400); **10,** The early zonate apex (×400); **11,** The vegetative apex (×400); **12,** The florat apex (×400); **C,** corpus; **CMZ,** central mother cell zone; **CO,** core; **CT,** cotyledons; **FP,** floral primordium; **LP,** leaf primordium; **M,** mantle; **PM,** pith meristem; **PZ,** peripheral zone; **PZE,** peripheral zone in the early stage of development; **T,** tunica. Arrows indicate lighter nuclei

Table 1 *Cytophotometric data of DNA, RNA and total proteins in the different zones of the shoot apex (Mean value ± SE)*

Stages	Zone*	DNA		RNA		Total proteins	
		Extinction value ($\times 10^{-3}$)	Content/ Nucleus	Extinction value ($\times 10^{-3}$)	Content/ Cell	Extinction value ($\times 10^{-3}$)	Content/ Cell
Plumular apex	T	6.185 ±0.191	1.051 ±0.032	2.234 ±0.170	2.636 ±0.181	11.356 ±0.191	11.685 ±0.196
	C	6.060 ±0.166	0.858 ±0.023	2.234 ±0.170	1.919 ±0.146	11.356 ±0.191	9.754 ±0.163
Early zonate apex	T	2.694 ±0.189	0.467 ±0.033	4.461 ±0.119	2.989 ±0.079	5.072 ±0.201	3.464 ±0.192
	CMZ	2.230 ±0.068	0.347 ±0.017	3.396 ±0.248	2.564 ±0.187	4.588 ±0.255	3.399 ±0.134
	PZ	3.042 ±0.177	0.529 ±0.032	4.223 ±0.182	3.428 ±0.147	5.441 ±0.188	4.416 ±0.152
Vegetative apex	T	3.042 ±0.177	0.459 ±0.027	4.461 ±0.189	2.189 ±0.058	6.810 ±0.166	3.342 ±0.081
	CMZ	2.346 ±0.116	0.309 ±0.015	4.104 ±0.194	1.953 ±0.97	6.310 ±0.204	3.156 ±0.102
	PZ	3.158 ±0.155	0.447 ±0.022	4.703 ±0.123	2.425 ±0.063	7.055 ±0.139	3.638 ±0.071
Transitional apex	M	3.509 ±0.119	0.562 ±0.019	5.812 ±0.185	2.413 ±0.077	8.100 ±0.173	3.363 ±0.072
	CO	3.158 ±0.155	0.477 ±0.023	5.441 ±0.188	2.208 ±0.076	7.970 ±0.198	3.235 ±0.081
Floret apex	M	3.628 ±0.158	0.514 ±0.022	7.450 ±0.198	3.341 ±0.089	7.060 ±0.037	3.165 ±0.016
	CO	3.274 ±0.116	0.386 ±0.013	6.685 ±0.191	3.028 ±0.086	6.810 ±0.166	3.085 ±0.075
	FP	3.866 ±0.194	0.547 ±0.027	8.364 ±0.197	3.750 ±0.088	7.450 ±0.198	3.551 ±0.089

C, Corpus; CO, Core; CMZ, Central mother cell zone; FP, Floral primordia; M, Mantle; PZ, Peripheral zone; T, Tunica

to the reproductive phase are debated. Buvat (1952) stated that "the central zone which became mitotically active during transitional stages is entirely responsible for the formation of bracts and inflorescence". Changes in apical zonation during transition from the vegetative to the reproductive phase reported in this study agree with the more commonly accepted interpretation that all zones become active and the entire apex is involved in the transformation.

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References

Buvat R 1952 Structure, evolution et fonctionnement du meristeme apical de quelques dicotyledones; *Ann Sci. nat. Bot.* **13** 596–656

Corson G E Jr and Gifford E M 1969 Histochemical studies of the shoot apex of *Datura stromonium* during transition to flowering; *Phytomorphology* **19** 189–196

Erickson R O, Sax K B and Ogur M 1949 Perchloric acid in the cytochemistry of pentose nucleic acid; *Science* **110** 472–473

Feulgen R and Rossenbeck K H 1924 Mikroskopisch Chemischer Nachweis einer Nucleinsäure Vom Typus der Thymonuclein-säure und die darauf beruhende Selektivfärbung Von Zelikernen in mikroskopischen Praparaten; *Zeitschr. Physik. Chem.* **135** 203–248

Gifford E M Jr and Tepper H B 1962a Histochemical and autoradiographic studies of floral induction in *Chenopodium album*; *Amer. J. Bot.* **49** 706–714

—— and —— 1962b Ontogenetic and histochemical changes in the vegetative shoot tip of *Chenopodium album*; *Amer. J. Bot.* **49** 902–911

Goyal S C, Jain S and Pillai A 1983 Localisation and semi-quantitative estimation of DNA, RNA and Proteins in the shoot apex of *Papaver somniferum* Linn; *Beitr. Biol. Pflanzen* **58** 67–75

——, Kavathekar K Y, Satija S and Pillai A 1980 Developmental anatomy of some oil-yielding plants. III. The seedling shoot apex; *Proc. Indian Acad. Sci.* **89** 7–13

Jacqmard A, Miksche J P and Bernier G 1972 Quantitative study of nucleic acids and proteins in the shoot apex of *Sinapis alba* during transition from vegetative to the reproductive condition; *Amer. J. Bot.* **59** 714–721

Jensen W A 1962 *Botanical Histochemistry*—Principles and Practice (San Francisco: W H Freeman & Co.)

Johansen D A 1940 *Plant Microtechnique* (New York: McGraw Hill Co.)

Kavathekar K Y and Pillai A 1979 Studies on the developmental anatomy of Ranales. VI. Histochemical studies of the shoot apex of *Delphinium ajacis* Linn. during transition of flowering; *Proc. Indian natn. Acad. Sci.* **B45** 577–585

Mauseth J D 1978a An investigation of the morphogenetic mechanisms which control the development of zonation in seedling shoot apical meristems; *Amer. J. Bot.* **65** 158–167

—— 1978b An investigation of the phylogenetic and ontogenetic variability of shoot apical meristems in the Cactaceae; *Amer. J. Bot.* **65** 326–333

Mazia D, Brewer P A and Alfert M 1953 The cytochemical staining and measurements of protein with mercuric bromophenol blue; *Biol. Bull.* **104** 57

Molder M and Owens J N 1973 Ontogeny and histochemistry of intermediate and reproductive apices of *Cosmos bipinnatus* var. "Sensation" in response to Gibberellin A_3 and photoperiod; *Can. J. Bot.* **51** 535–551

Nougaréde A 1956 Organisation et fonctionnement du méristème apical des végétaux vascularires; in *Travaux dedies au Hucien Plantefol* (Paris: Masson et Cie) 520 pp

Orr A R 1978 Inflorescence development in *Brassica campestris* L.; *Amer. J. Bot.* **65** 466–470

Rogan P G and Smith D L 1974 The development of shoot apex of *Agropyron repens* (L.) beauv.; *Ann. Bot.* **38** 967–976

Shah C K, Bhatt P N and Patel K R 1975 Use of photoresistor for the measurements of light absorption in cytophotometer; *Indian J. expt. Biology* **13** 505–507

Tepper H B and Gifford E M Jr 1962 Detection of ribonucleic acid (RNA) with pyronin stain; *Stain Tech.* **37** 52