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Shoot Apical Organization in Seedlings of some Indian Pulses [1])

By

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With 14 Figures

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Summary

Ten species of pulses have been worked out to study comparative organization of the shoot apical meristem in their seedlings of about 8—10 plastochrons age (4—5 visible leaves and 4—5 leaf primordia at the shoot apex). Only two species show two tunica layers and the others show only one. Based on the staining reaction of the cells, only *Vigna aconitifolia* shows the presence of cytohistological zonation. In the 'long' and 'short' apices organo-histogenic zonation is evident. Leaf initiation may occur by periclinal divisions either in T_1, T_2 or the outermost corpus layer. But in some cases the leaf buttress is formed by only enlargement of the cells of the one or two superficial tunica and corpus layers at the site of the leaf initiation. Yet another case of the early sign of leaf initiation is found by the presence of more mitotic activity in the deeply seated cell layers. This shows that the periclinal divisions in superficial layers are not obligatory for the initiation of a leaf. Internodal and cortical differentiation starts either soon after the initiation of the leaf or when a very small leaf buttress is formed.

1. Introduction

Much attention has been devoted towards the root apical organization studies of pulses (see Clowes 1961). But the shoot apical organization of the pulses is not well known (Reeve 1948, Mitra 1950, Sun 1957, Bostrack & Struckmeyer 1964, Ball & Soma 1965, Pillai & Sukumaran 1969). In our programme to undertake the development and ana-

[1]) pulses = Hülsenfrüchtler (Anmerkung des Editors)

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tomical investigation in most important pulse plants of India, it was thought worth surveying the shoot apical organization of this group of plants. This is the second contribution in a series on anatomical studies on pulses (PATEL, SHAH & SUBBAYAMMA 1975). Ten important pulses have been selected for the present study.

2. Materials and Methods

Seeds of ten important edible pulses were obtained from various sources (Table I). The seeds were grown in the Botanical Garden of the Sardar Patel University after the first rains in the month of July, 1972. The shoot apices of the plants having about 4—5 visible foliage leaves were collected and fixed in FAA (SASS 1958). The species having more trichomes were gently shaved near the stem tip in such a way that apical meristem and young leaves were not injured. This was essential to facilitate fixation and infiltration. The materials were dehydrated, infiltrated using customary methods. Longisections, 6—8 μm thick, were stained with tannic acid — ferric chloride (FOSTER 1934), REGAUD's hematoxylin and fast green combinations. Some of the slides were stained using SHARMAN's schedule (1943) with safranin — O as a substitute of orange G.

3. Observations

As the present investigation deals with the comparative shoot apical organization in various pulses (Table 1), we have not emphasized the structural changes that occur during a single plastochron. However, general description of the shoot apex is based on observations of apices exhibiting important organogenic stages. About 50 shoot apices of each species are observed. The shoot apices studied are about 8—10 plastochrons old (4—5 visible foliage leaves and 4—5 young leaf primordia at the apex are present).

3. 1. General Organization

The shoot apex shows a tunica-corpus organization, and in most of the investigated species the cytohistological zonation based on staining affinity is absent. The number of tunica layers varies from one to two. In *Cicer arietinum* (Fig. 1) and *Lathyrus sativus* (Figs. 3, 8) two tunica layers are present, whereas in *Vigna radiata*, *V. angularis*, *V. aconitifolia* (Fig. 9); *V. unguiculata*, *Pisum sativum* (Figs. 2, 7), *Lens culinare*, *Glycine max* and *Lablab purpureus* (Figs. 4, 6) only one tunica is present (Table I). A massive corpus is envaloped by one or two tunica layers. Stratification in the corpus cell layers is found in *C. arietinum* (Fig. 1), *V. unguiculata* and *L. purpureus* (Fig. 6). In rest of the species corpus is not stratified.

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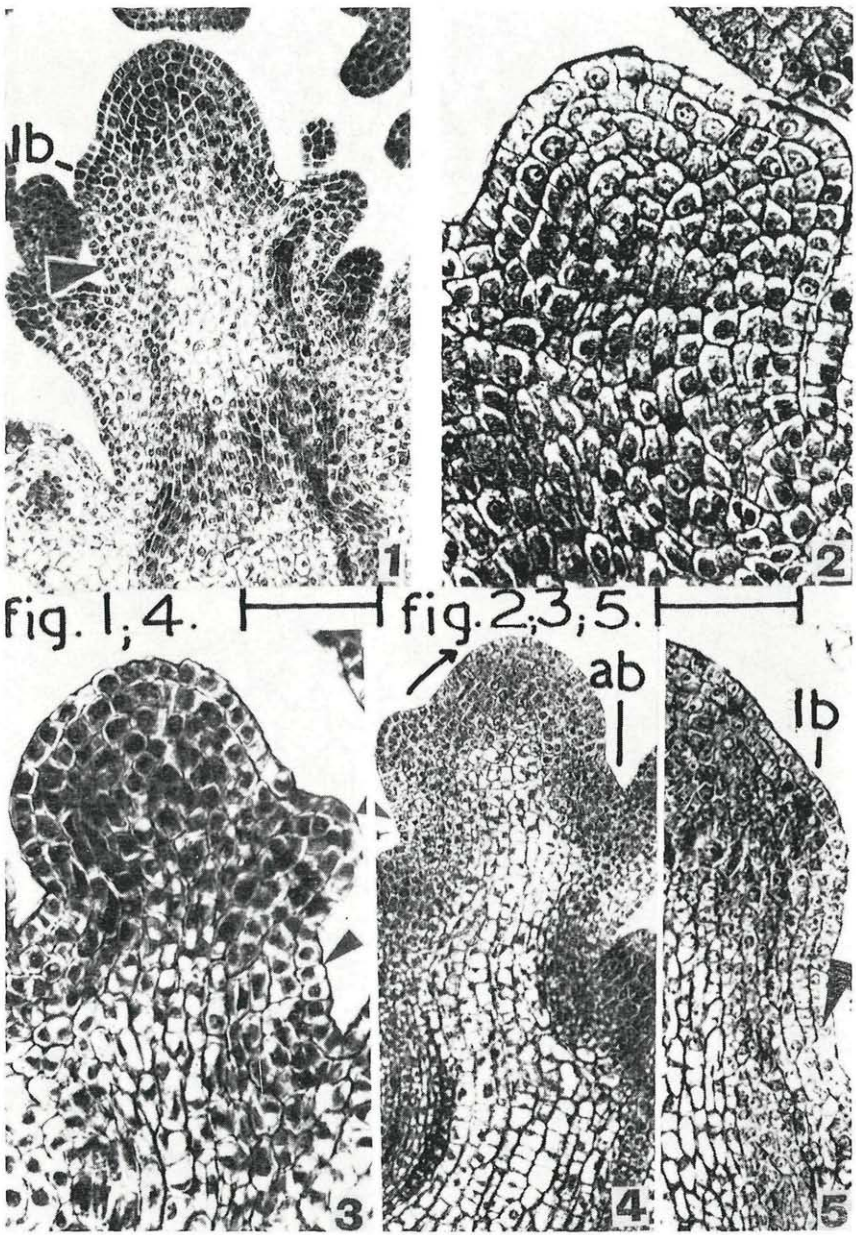

Figs. 1—5. Longisections of the shoot tips. (ab = axillary bud; lb = leaf buttress; arrow-head = differentiating internode; arrow: the direction of curvature of the apex). — 1. *Cicer arietinum*. — 2. *Pisum sativum*. — 3. *Lathyrus sativus*. — 4. *Lablab purpureus*. — 5. *Vigna unguiculata*. Scale 1,4.—100 μm; 2, 3, 5. — 50 μm.

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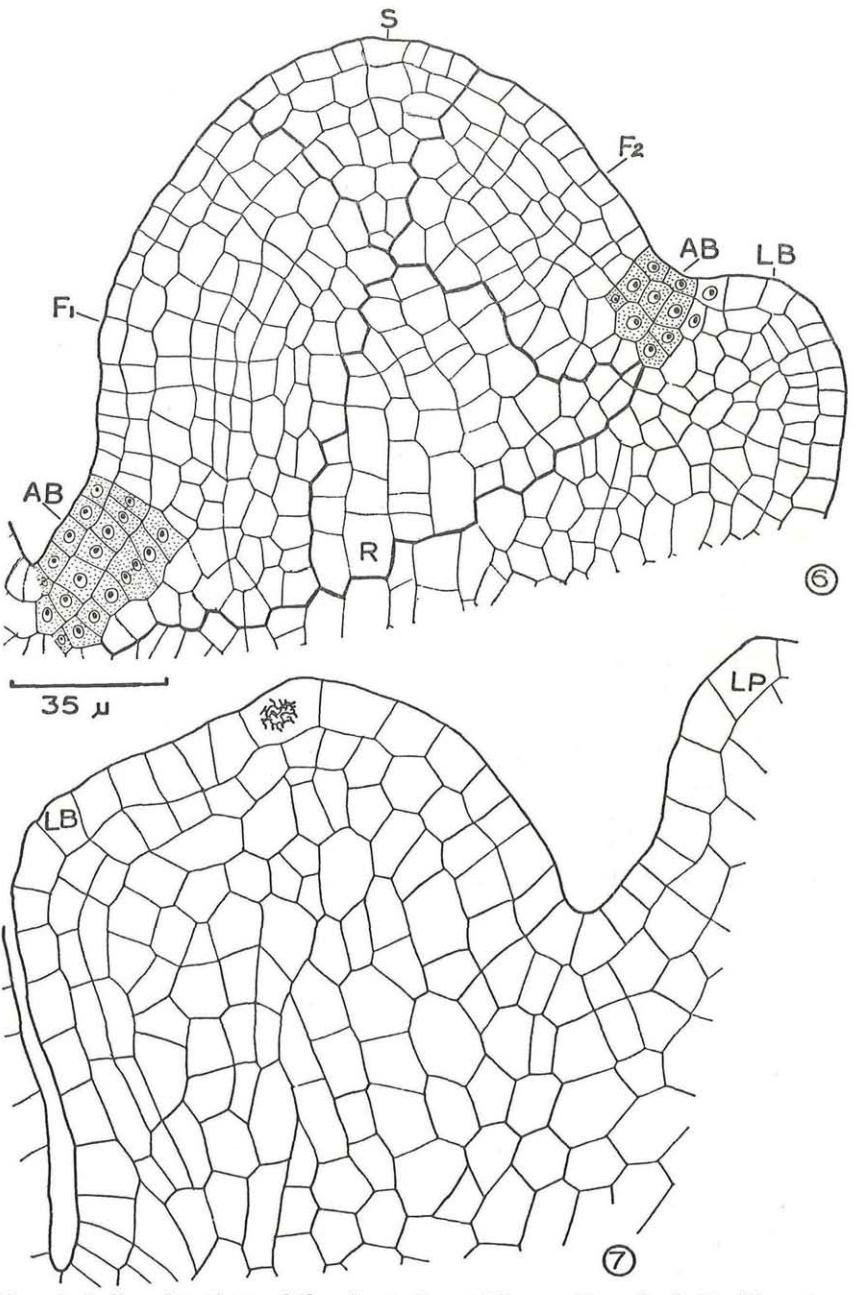

Figs. 6, 7. Longisections of the shoot tips. (AB = axillary bud; F_1, F_2 = two flanks of the shoot apex, also see explanation in the text; LB = leaf buttress; LP = leaf primordium; R, S = cells at the pith and summit regions of the shoot apex respectively). — 6. *Lablab purpureus* showing the organo-histogenic regions. — 7. *Pisum sativum* short type of apex, note the mitotic figures in one of the cells of the central or summit region of the apex.

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3. 2. Types of shoot apices

Two main types of apices, short and long, are recognized. In the literature this terminology has not been used with reference to dicotyledonous shoot apices. SHARMAN (1947) has described three patterns of shoot apices in grasses based on their height. In the present investigation the apices in which the height of the apex exceeds its width and/or apices having more than one leaf primordia shorter than the shoot apex are considered 'long' ones, and those in which the height is either less or nearly equal to the width of the shoot apex are 'short' ones. The long apices are high dome-shaped, whereas the short apices show low dome convexity. During various plastochronic phases the general 'long' or 'short' nature of the apex does not alter. The long apices, furthermore, are of two types: (i) in which the new leaf is initiated only after the youngest formed leaf either reaches or exceeds the height of the shoot apex as in *V. aconitifolia*, and (ii) in which a new leaf initiates and develops even before the last formed leaf is either in the form of a small buttress, or a small primordium which has not yet overtopped or reached the height of the shoot apex (Figs. 1, 3, 11, 12). In some of the species 3—4 such leaf primordia are found which are shorter than the shoot apex (Figs. 1, 4, 11, 12). In the shorter type, two kinds of apices can be recognized. In the first the width and height ratio is almost equal to one. In this type the leaf develops in the peripheral part of the apex, and the central region of the apex is not directly engaged in leaf genesis. The new leaf is initiated only when the last formed leaf overtops the height of the apex as in *P. sativum* (Figs. 2, 7). In the second type of the short shoot apices, as in *L. culinare*, the apex is low-domed and some of the cells of central region are involved in the development of the leaf buttress.

3. 3. Zonation

In most of the species the cytohistological zonation, based on staining affinity, is absent. However, *V. aconitifolia* shows cytohistological zonation wherein central meristem, peripheral meristem and rib meristem are differentially stained (Fig. 9). The central meristem is lightly stained as compared to the peripheral meristem. Cells of the rib meristem exhibit more vacuolation and hence very lightly stained (Fig. 9, peripheral stippling in the rib meristem region indicates vacuolation). However, the presence of rib meristem is evident even in the species where differentiation between the central and peripheral region is not evident, based on staining reaction (Figs. 1, 3, 4). Though the cytohistological zonation cannot be recognised in most of the species, the organo-histogenic zonation can be identified (Figs. 6, 10). This idea of organo-histogenic zonation has been followed after DENNE (1966) who has divided the shoot apical meristem of *Trifolium repens* into five regions and named as: (a) Region-S which includes the cells at the

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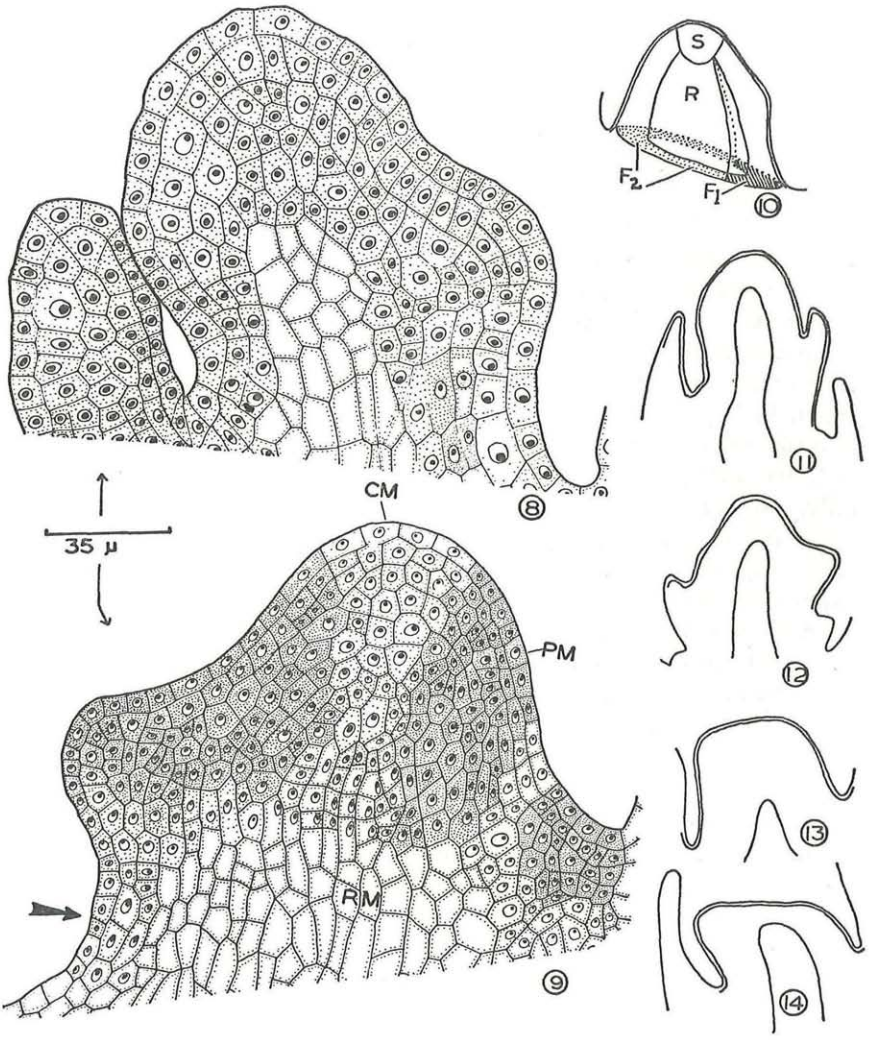

Figs. 8—14. Shoot tips of pulses. (CM = central meristem; F_1, F_2 = two flanks of the shoot apex, see text for details; PM = peripheral meristem; R = pith region; RM = rib meristem; S = summit region of the apex). — 8. *Lathyrus sativus*. — 9. *Vigna aconitifolia*. — 10. Schematic representation of organo-histogenic zonation in the apex. — 11, 12. Schematic representation of long type of apices showing more than one leaf primordia shorter than the shoot apical height. — 13, 14. Short types of shoot apex shown in schematic out-line drawings.

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summit of the apex; (b) Region-F_1 which includes the flank or peripheral part of the apex which is concerned in the formation of the next leaf primordium; (c) Region-F_2 which includes a peripheral part which would form the next leaf primordium after the formation of a previous leaf primordium in region F_1; (d) Region-R which is rib meristem; and (e) Region-A which is the axillary region of the youngest leaf primordium which would form the axillary bud. Similar distinctions of regions could also be made in the pulse apices. Region-F_1 can be identified either by the presence of narrow, small cells or disturbed arrangement of the cells, indicating the preparation of the site for the next leaf formation (Fig. 6). On the rest of the flanking region of the apex Region F_2 is present which can be recognized either by large cells or less frequency of mitotic activity. The cells in the Region-S are not infact related directly to the formation of the lateral organs, in most of the cases, but they are histologically active and contribute to the region F_1, F_2 and R. Region-R shows vacuolated cells which differentiate into the ground tissue of the pith. However, we do not consider Region-A of DENNE (1966) as a part of the shoot apex. As the leaves are formed spirally, and not in the opposite pairs, we do not consider the opposite flank of the shoot apex to the Region-F_1 as the Region-F_2. Because in a median longisectional view it is difficult to locate the site of a future leaf initiation (other than the one which is likely to be formed at F_1 Region). Hence, we consider the entire flank but the F_1 Region as F_2 Region. It is interesting to note that mitotic figures were found, sometimes, in the Region-S in *C. arietinum* and *P. sativum*.

3. 4. Leaf initiation

In the species showing two tunica layers, *C. arietinum* and *L. sativus*, the leaf initiation occurs by periclinal divisions in the cells of T_2 layer. However, in *L. sativus*, sometimes, periclinal divisions are absent in T_2 for the initiation of the leaf, but precocious leaf trace is found towards the site of the leaf initiation. Sometimes, in *C. arietinum* a small bulge is formed at the site of the leaf initiation without any periclinal divisions in T_2 cells. Instead, the outermost corpus cells show more mitotic activity. In *V. unguiculata* the site of the new leaf formation can be recognized by more frequent mitotic activity in 2—3 corpus layers on the flank of the shoot apex. It is interesting to note that even with such a high mitotic activity at the site of leaf initiation a leaf buttress is formed very late. This is, probably, due to the delayed growth of the cell derivatives at the site of leaf initiation. Sometimes, the situation of the leaf initiation is indicated by the enlargement of T_1 and outermost corpus layer's cells. In such cases the early differentiation of the leaf is not caused by periclinal divisions in superficial layers of the apex. In *P. sativum* the leaf initiation starts with more periclinal divisions in the outermost corpus layer.

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3. 5. Differentiation of cortex and internode

The early differentiation of the internode can be observed in *V. anguiculata* as soon as a small leaf buttress is formed. The cells of the abaxial side of this buttress show light stainabilty and vacuolation (Fig. 5). The internode is more evident even before the leaf buttress grows to a prominent leaf

Table 1
Comparative account of shoot apical organization in ten pulse species

Species	Source	T	CS	CHZ	SA	LP
Cicer arietinum L.	IARI	2	+	—	L	3—4
Glycine max L.	BACA	1	—	—	S	1
Lablab purpureus (L.) SW. (*Dolichos lablab* L.)	BACA	1	+	—	L	3—4
Lathyrus sativus L.	IARI	2	—	—	L	2—3
Lens culinare Medic. (*Lens esculenta* Moench.)	IARI	1	—	—	S	1
Pisum sativum L.	IARI	1	—	—	S	1
Vigna aconitifolia (Jecq.) Marechal (*Phaseolus acontifolius*)	ACJ	1	—	+	L	1
Vigna angularis (Wills.) Ohwi & Chashi (*Phaseolus mungo* L.)	UPIA	1	—	—	L	2—3
Vigna unguiculata (L.) Walp. (*Vigna catjang* L., Walp.)	IARI	1	+	—	L	2—3
Vigna radiata (L.) Wilczek (*Phaseolus aureus* Roxb.)	IARI	1	—	—	L	1—2

Abbreviations and explanations: T = number of the tunica layers; CS = stratification of corpus cell layers; CHZ = cytological zonation (+ = present, — = absent); SA = type of the shoot apex (L = "long" type, S = "short" type); LP = number of leaf primordia at shoot apex which are shorter than the shoot apex. Sources: ACJ = Agricultural College, Junagarh, Gujarat (India); BACA = Bansilal Amritlal College of Agricultur, Anand, Gujarat; IARI = Indian Agricultural Research Institute, Delhi; UPIA = Utter Pradesh Institute of Agricultural Sciences, Kanpur (India).

primordium (Figs. 3, 4, at arrow). The early internode consists of a protoderm layer, 2—3 rows of cortical ground meristem cells, procambial cells, and pith (Fig. 1, at arrow). The cortical ground meristem layers of the internode increase in number by periclinal divisions in second and third subsurface layers at fourth to sixth internodes below the shoot apex in different species. Further differentiation of cortex and pith is in agreement with that reported in brinjal and chilli (Patel & Shah 1971).

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The files of the pith cells can be traced in straight lineages from the rib meristem down to the lower part of the axis as in *V. aconitifolia*. But sometimes it is found that these files of cells cannot be traced from the rib meristem down to certain number of internodes in straight files as in *L. purpureus* (Fig. 4), and *L. sativus* (Fig. 3). Probably, this type of curvature is brought about by differential elongation on the two flanks of the shoot tip. Such curvature of the shoot apex occurs on the opposite side of the new leaf development site (Fig. 4, in the direction of the arrow). So, curvature of the pith would also be in the direction of arrow at that node. Subsequently, a new leaf will develop on the other site of the remaining part of the flank of the shoot apex during which the shoot apex will be pushed towards the left side (as in Fig. 4), and hence the pith at that node will be curved towards the left. Table 1 gives the general idea of the shoot apical organization in investigated species.

4. Discussion

If the total volume of an apical meristem is compared with the volume of the plant, it is very very negligible, even then the importance of such a tiny, perpetually dividing tissue is tremendous (Romberger 1963). This is the centre of organogenic and histogenic activities of the plant body, and hence it has been rightly studied more extensively since the latter part of the ninteenth century (Gifford 1954). The various species so far investigated show the presence of two or three tunica layers (Gifford 1954, Pillai & Sukumaran 1969). However, in the present investigation it has been found that in eight out of ten species only one tunica layer is present, whereas only two species show two tunica layers. Tunica is reported to be two layered in cultivar 'Hawkeye' (Bostrack & Struckmeyer 1964) and 'Marosoy' (Krause 1971) of *Glycine max*. However, only one tunica layer is present in the cultivars 'Junagarh 202' and 'Clarke' of *G. max*.

The shoot apex of many angiosperms shows cytohistological zonation (Gifford & Corson 1971). Nevertheless, there are a number of instances where the angiosperm species are devoid of such zonation based on colour reaction with stains (see Patel & Shah 1972). In *C. tetragonoloba* cytohistological zonation in the shoot apex is reported during the middle and maximal phases of the plastochron (Pillai & Sukumaran 1969). In the present investigation only *V. aconitifolia* shows the presence of cytohistological zonation. This shows that the cytohistological zonation is apparently not related to the functional activity of the shoot apex (Patel & Shah 1972). Nevertheless, physiological zonation must be present in the apex for its organogenic and histogenic activities. Denne (1966) has shown, in *Trifolium repens*, this type of zonation based on topography and behaviour of the cells in the different regions of the shoot apical meristem. This is a sort of physiological attribution to various regions of the apex. We have

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also found such organo-histogenic zonation in the species where cyto-histological zonation is absent.

SHARMAN (1947) showed three types of shoot apices in grasses based on height and number of small leaf primordia at the shoot tip — short, medium and long. But, there is no attempt made by any worker in this field, as far as we are aware of, to classify dicotyledonous shoot apices into long and short types. We have used these criteria for the classification of the apices of pulses.

Leaf initiation may occur even without the early periclinal divisions in the tunica or outermost corpus layer in the four investigated species. FOARD (1971) has also shown in wheat that initiation of the bulging of the leaf buttress can be caused without periclinal divisions in tunica or corpus layers when the plant is treated with X-rays. In *Sorghum vulgare* SHAH & PATEL (1974) reported the similar observation for the initiation of some chaff members of the spikelet.

ABBE & POLLOCK (1946) showed, in *Anacharis*, that the cortical parenchyma cells have their origin from the rib meristem. The first sign of cortical development can be found in the fourth to thirteenth internode (BARTHELMESS 1935, ABBE & POLLOCK 1946). In *Solanum tuberosum* SUSSEX (1955) reported the origin of cortical tissue from the tunica and outer corpus layer of the apical meristem. In brinjal and chilli the earliest recognizable internode and cortex differentiate as soon as the leaf buttress appears at the flank of the shoot apex (PATEL & SHAH 1971). The present investigation is in agreement with these observations.

We are grateful to Professor J. J. SHAH for his encouragement. One of us (J. D. P.) thanks the University Grants Commission for a Sr. Research Fellowship during the tenure of which the present investigation was carried out.

5. Zusammenfassung

Die Vegetationspunkte der Keimlinge von zehn wichtigen indischen Leguminosen (Alter 8—10 Plastochrone, d. h. 4—5 sichtbare Blätter und 4—5 Blattprimordien) werden vergleichend untersucht. Nur zwei Arten weisen zwei Tunica-Schichten auf, die übrigen nur eine einzige (vgl. Tab. 1). Nur bei *Vigna aconitifolia* ist auf Grund des Verhaltens gegenüber Farbstoffen eine cytohistologische Zonierung erkennbar. Eine organo-histogenetische Zonierung ist bei den Sproßscheiteln vom Typ „lang" und „kurz" deutlich. Die Blattbildung kann mit periklinen Teilungen in der T_1-, der T_2-Schicht oder der äußersten Corpus-Schichten einsetzen. Gelegentlich bildet sich der Blatthöcker jedoch nur durch Vergrößerung der Zellen einer oder beider Tunica-Schichten und des Corpus. In einem anderen Falle hinwieder erwies sich erhöhte mitotische Aktivität tiefgelegener Zellen als frühes Anzeichen der Anlage eines neuen Blattes. Perikline Teilungen oberflächlicher Zellschichten sind somit nicht unbedingtes Erfordernis für die

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Blattbildung. Die Differenzierung in Internodium und Rinde setzt bald nach dem Auftreten der ersten Anzeichen der Blattbildung ein oder sobald sich ein sehr kleiner Blatthöcker gebildet hat. (Transl. by the editor.)

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