DEVELOPMENTAL ANATOMY AND HISTOCHEMISTRY OF CERTAIN GYMNOSPERMS

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BABY CHACKO

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To My Parents

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

PILANI, RAJASTHAN

CERTIFICATE

This is to certify that the thesis entitled "Developmental Anatomy and Histochemistry of Certain Gymnosperms" and submitted by Mr. Baby Chacko, ID No. 72S87001 for award of Ph.D. degree of the Institute, embodies original work done by him under my supervision.

Date 17-1-1976

(S.K. PILLAI)
Professor
Department of Biological Sciences

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CONTENTS

CHAPTER I

INTRODUCTION

The intricate problem of development of higher plants has attracted attention for a long time. But it still remains high and distant. The recent attempts to tackle the problem from different angles and levels, have made noteworthy headway. But the pursuit is to be continued.

If the term 'development' conceives the idea of a series of changes which an organism goes through during its life cycle, it should comprise of two components, one of growth, another of differentiation. The former is a quantitative expression showing visible, but irreversible changes, while the latter is a qualitative expression resulting in structurally dissimilar cells, tissues or organs. The study of differentiation at the levels of cells, tissues and organs composes the field of developmental anatomy.

The present investigation is mainly in this line on members of an evolutionarily important group, that comprises half of the forest wealth - the gymnosperms - a group that exhibits many characteristics. Accordingly Romberger (1966) stresses, "The coniferous tree, an important crop plant of our overcrowded world, has many interesting

developmental characteristics which recommend it as a useful experimental organism in developmental biology."

A few of the developmental phenomena of plants, such as vascular differentiation, organ development, dormancy strobilar initiation, are attempted here. The results are compiled in different sections, the first of which comprises a survey of the organisation of shoot apices in several members of Indian gymnosperms starting from the primitive to the advanced. This has helped to draw the direction of evolution of the shoot apical organisation in the different members of the group. In addition to the apical structure, the growth periodicity of the buds of two conifers that contribute to the forest wealth of Himalayas is also studied. Much work on this aspect has not been done in Indian plants.

Attention was given to the "crown", a peculiar anatomical structure reported in many conifers. But its detailed study was left unattempted by earlier workers. Its structure, development, and periodic changes are followed to understand its possible role.

While the apical meristem governs the primary growth of the aerial structures of most of the gymnosperms, an intercalary meristem is also active in Ephedra. Effort has been made to study its development to understand the possible time of activity and later destiny of the intercalary meristem in Ephedra foliata. The embryonic structure, and seedling anatomy of a conifer, Picea smithiana are also

attempted.

Lastly, a two directional approach to the process of strobilar initiation in Ephedra foliata is attempted. The manifold changes involved in the meristem of the shoot during its transition to floral initiation have been studied anatomically and histochemically.

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CHAPTER II

REVIEW OF LITERATURE

Though the importance of the shoot apex in higher plants was realised in the mid-eighteenth century (Wolff , 1759) the interpretations of the structure and mode of growth of the apical meristem came to be postulated on a general basis only after nearly a century. The apical cell theory proposed by Nägeli in 1844 based on the study of mosses and algae was later tried to be applied to higher plants also. He placed all the meristematic importance to a single cell. Hofmeister (1851, 1857) supported this concept by reporting a single apical cell in some gymnosperms and angiosperms. Hofmeister's observations in gymnosperms remained unchallenged till the work of Strasburger (1872) and Groom (1885). Strasburger was not convinced of a single apical cell in his observations of gymnosperms like Cycas, Ginkgo, Cryptomeria, Taxus, Ephedra, etc. He noticed a discrete outer layer called dermatogen and interpreted their organisation based on Histogen theory.

Earlier Hanstein (1868) had superseded the apical cell theory of Nägeli (1844) with his Histogen theory. Based on his studies on 46 genera of angiosperms, Hanstein (1868) evolved his theory, which maintained that the shoot

apex in angiosperms consisted of an inner core of irregularly arranged cells covered by a variable number of mantle like layers. He proposed that each layer and the core derived from a distinct initial or a group of initials, be called the histogens. He gave the terms 'dermatogen', 'periblem' and 'plerome' for these distinct histogens. The idea of predestination in the histogen theory invited a lot of criticism.

Strasburger (1872) used a new terminology for his interpretation of gymnosperm apices. He preferred the term protoderm to Hanstein's dermatogen. In later years these dissenters were resented by some Botanists. They adhered to the Nägelian concept of a single cell theory. The latest report of support to the apical cell theory is that of Johansen (1950). He reported an apical cell with three cutting faces to be active in the early stage of embryonic development in Pinus.

Strasburger's view was supported by Warming (1877) with his observations on Ceratozamia longifolia and Cycas circinalis and Bower (1844) on Cycas seemani.

Contrary to the idea of observing the meristem based on morphology alone, Koch (1891) studied it from the cytological point of view too. He gave a detailed account of the cytohistological zonation in the shoot apical meristem of many conifers and Ephedra. He considered the apex to be formed of two well defined regions, an outer peripheral

mantle composed of densely cytoplasmic cells and an inner core of larger, vacuolated and dividing cells. These zones did not however correspond to the histogens of Hanstein (1868).

A novel interpretation of apical organisation and growth was given by Schmidt (1924) laying emphasis on two tissue zones, viz., Tunica, an outer sheath, covering an inner core called corpus, under the title Tunica-corpus theory. While the apical cell theory and histogen theory served for the shoot and root apices of gymnosperms and angiosperms, Schmidt's theory was applicable only to angiosperm apices.

Topographical and cytological details that served as criteria for the interpretation of the meristem till 1930, were supplemented with physiology and developmental morphology in later years. The works of Louis (1935), Barthelmess (1935) and Kaplan (1937) were mainly centred on organs and tissues of shoot development from the relatively undifferentiated tissue.

The choice of Ginkgo for his studies on shoot organisation enabled Foster (1938) to evolve a theory of cytohistological zonation. He combined the tunica-corpus concept with the cytohistological features of the apical tissues as related to their histogenic role. Elaborating the idea conceived by Koch (1891), Foster distinguished one zone of tissue from another based on (1) cell size and

degree of vacuolation, (2) nuclear volume, (3) staining characteristics, (4) frequency of cell division, (5) relative cell wall thickness, and (6) orientation of planes of cell division.

Foster recognised five zones in Ginkgo apices, viz., (a) apical initials, (b) central mother cell zone, (c) transition zone, (d) peripheral zone, and (e) rib meristem.

While many of these workers believed that all tissues of the shoot are ultimately derived from the superficial initials, one French anatomist Camefort (1950, 1951) expressed some doubts regarding the participation of the apical initials and central mother cells in the formation of gymnosperm apex. He considered these two zones together as a 'zone apicale' as they exhibited very little mitotic activity. The peripheral zone was recognised as 'anneau initial' and the rib meristem zone by the term 'meristeme medullaire'. The same terms had been used for angiosperms by Plantefol (1947).

After the emergence of the theories of Foster and Camefort there was an overwhelming resurgence of interest in the ontogeny of the shoot apex of vascular plants with special reference to gymnosperms. Ontogenetic studies of the shoot apex of gymnospermous plants were made in various members by Allen (1946, 1947a,b), Ball (1956a,b), Chouinard (1959), Cross (1939, 1941, 1942, 1943a,b), Curtis

and Popham (1972), Foster (1938, 1939, 1940, 1941a,b, 1943, 1949), Gifford (1943, 1961), Gifford and Wetmore (1957), Griffith (1952), Hanawa (1966, 1967), Jackman (1960), Johnson (1939, 1944a,b, 1950), Kemp (1943, 1959), Konar (1960), Kupila and Gifford (1963), Paollilo and Gifford (1961), Parke (1959), Pillai (1963a,b, 1964), Pillai, A. (1962, 1963), Pillai and Pillai (1974), Sacher (1954), Shah and Thulasy (1967), Singh (1961), Spurr (1949), Sterling (1945a,b, 1946, 1958), Taillandier (1965), Tepper (1963, 1964, 1966), etc.

Information on these aspects was compiled by a few investigators from time to time. Popham (1951) reviewed the work till the time and classified the accumulated information on shoot apices of vascular plants into seven categories, the gymnosperms being confined to 3 categories. Johnson (1951) arranged the accumulated data on gymnosperms familywise and tried to show some common points of similarity within the group. Gifford and Corson (1971) treated the gymnosperms as separate in their review of the shoot apex in seed plants.

A few studies of gymnosperm apices were concerned with the allocation of the stage at which apical meristems are distinguished in the young embryo, and the establishment of the primary vasculature. Allen (1947b) studied the embryogeny of Pseudotsuga and reported the zonation of the shoot apex to be clear only after 3-4 months. Spurr (1949) noticed in the mature embryo of Pinus strobus the nuclei of

the peripheral cells of the shoot apex to be relatively large and lightly staining, but did not attribute any zonation. Fosket and Miksche (1966) noticed in Pinus lambertiana the zonation to appear by 7th day after germination. Gregory and Romberger (1972) for the first time gave the distinction of a zonated apex to the shoots of immature embryos of Picea abies.

Many of the earlier studies of apical organisation of gymnosperms were made on materials collected once and slight fluctuations were noticed in the organisation of the apex of the same species. Such data were considered inadequate. Hence studies were made on material collected during different seasons and from plants of different ages. Variations in the apical organisation were noticed in branches of different ages and vigour by Cross (1939) in Taxodium distichum, and by Gifford (1943) in Ephedra altissima. Owston (1969) noticed variation in the organisation of the apices collected from different heights of the same plant of Pinus strobus.

Periodic studies of the apex made by Griffith (1952) on Araucaria, Sacher (1954) on Pinus lambertiana, Camefort (1956) on Picea excelsa, Frampton (1960) on Larix decidua, Singh (1961) on Cephalotaxus drupacea, Pillai (1963a) on Podocarpus gracilior showed that there is a definite period of dormancy in these plants. Instead, Araucaria (Pillai, 1964), Cupressus sempervirens (Pillai, 1963b) and Thuja orientalis (A. Pillai, 1963) lacked such a period of

dormancy in the annual cycle.

Owens and Molder (1973) followed the change in DNA and mitotic activity in the vegetative apex of Douglas fir during the annual growth cycle. This is the only histochemical study conducted for a full year.

The biochemical differences existing between the zones of the shoot meristem were traced out in some gymnosperms by histochemical methods. Paolillo (1963) analysed the apices of Ephedra altissima using histochemical methods. Van den Born (1963) used histochemical techniques to localise the enzymes in the different zones of the shoot apex of Picea glauca.

Fosket and Miksche (1966) studied the activity of acid phosphatase, succinic dehydrogenase, and protein-bound sulphydryl groups during the development of the seeds of Pinus lambertiana. Owens (1969) noted in Douglas fir an increase of succinic dehydrogenase during lateral bud initiation. Durzan et al (1971) made an extensive study of changes in the metabolites of dormant as well as germinating seeds of Pinus banksiana. Cecich et al (1972) conducted a cytophotometric study of nucleic acids and protein fluctuations in the shoot apex of Picea. Riding and Gifford (1973) studied the changes in cell constituents of Pinus radiata seedling shoot apices, and provided evidence to show that the apex as a whole is metabolically active during vegetative growth.

Studies on the apical meristem during its transition to flowering have been made in many angiosperms. But this has rarely been extended to gymnosperms. It is probably because of the longer duration of development of the strobili. Hejnowicz (1957) reported the apices of vegetative and ovulate strobilus of Chamaecyparis pissifera to have the same organisation. Gifford and Wetmore (1957) could not notice any discernible difference except in size and form in Larix decidua. In their comparative study of vegetative and strobilar apices Kemp (on Torreya 1959) and Gifford and Mirov (1960) in Pinus ponderosa and Gifford (1961) in Pinus murrayana, Picea ericoides could not trace any noteworthy difference in organisation.

Loze (1965) noticed in Taxus baccata the ovule bearing shoot apex resembling the vegetative shoot until nucellar formation. Owens and Smith (1964) studied the transitional apices of Pseudotsuga douglassi and observed no more remarkable changes during the transition than those that prevailed in the vegetative apex during different seasons.

Taillandier (1966) traced, by histochemical methods, the seed cone development in Pinus maritima and observed a gradual disappearance of the zonation and activation of the apical axial zone during the initiation of sterile and fertile bracts.

In Cupressus arizonica and Thuja plicata, the effect of gibberellic acid on development of cones was studied by Owens and Pharis (1967, 1971). During the transition from

vegetative to reproductive, a precocious branching was noticed in the former, followed by a three-fold increase in mitotic frequency.

Taillandier (1967) successfully used labelled thymidine to localise the synthesis of DNA in the transitional apex of Pinus maritima and noticed the distribution of the labelled compound uniformly in the meristem from the time of inception of last protective scales.

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CHAPTER III

MATERIALS AND METHODS

All the samples for the present study were collected from plants growing in their natural habitats. Shoot apices of *Cycas circinalis* Linn. were collected from Palai, Kerala. Materials of six species of *Pinus* viz., *P. roxburghii* Sarg., *P. wallichiana* A.B. Jacks, *P. insularis* Endl., *P. gerardiana* Wall., *P. massoniana* Lamp and *P. pseudostrobus* were collected from plants growing in the Forest Research Institute Nursery, Solan. Shoot apices of *Abies pindrow* Spach. and *Taxus baccata* Linn. were collected from plants growing at Khadrala, 80 miles up from Simla. Monthly collections of shoot portions of *Picea smithiana* (Wall.) Boiss. and *Cedrus deodara* (Roxb.) Loud were made from Simla during the period 1972-73. In all these cases apices from the same height of the different plants were chosen to avoid organisational differences which were reported by Owston (1969).

Specimens of *Ephedra foliata* Boiss. were procured from plants growing around Pilani.

The seedlings for the study were raised by sowing seeds in vermiculite. Subsequently they were transferred to the soil. In some cases a cold treatment at about 5°C for 25 days had to be given for effecting germination.

The plants were watered with distilled water once in two days and Hoagland's solution was supplied once in a

week. To avoid disparities, seedlings of uniform length of the radicle were taken as a lot from the petri dish and transplanted in soil.

The general fixative used was F.A.A. Riding's (1970) modification of F.A.A. gave better results. It was used for general and Feulgen staining, periodic acid-Schiff's reaction, protein and histone staining. For the enzyme localisation of acid phosphatase and peroxidase, Wolman's fixative was used as suggested by Riding (1970), but was found unsatisfactory. Attempts with fresh materials, however, gave good results. Processing was through TBA series and paraffin and sections were cut at 7-15 μ thickness. Softening of the tissue was needed for pine buds and was carried out with treatment in Teepol.

Northan's variation of Foster's Tannic acid-Ferric chloride-Safranin-Fast green Staining Schedule (Johansen, 1940) and crystal violet - erythrosine combination (Johansen, 1940) were adopted for general staining.

Insoluble polysaccharides were determined with periodic acid - Schiff's reaction (Jensen, 1962). Materials not treated with periodic acid served as controls. Riding's modifications of Feulgen staining for DNA, and Azure B staining for RNA and DNA gave good results. Control for RNA was given by Tepper and Gifford's (1962) method.

Mazia et al's (1953) staining schedule was used for total protein with mercuric chloride - Bromophenol blue.

Fast green FCF was used for histone staining following the method of Alfert and Geshwind (1953). Sections hydrolyzed with TCA followed by Trypsin digestion served as control (Pearse, 1960).

Lignin localisation was done following Jensen (1962) using Phloroglucinol. Pectin could be noticed with his method using Ruthenium red.

Acid phosphatase localisation was achieved using a modified method of Gomori's lead sulphide procedure (Benes and Opatrna, 1964). Sodium glycero phosphate was omitted from the incubation medium for the control. Peroxidase was traced with benzidine and H_2O_2 following the method of Kiszely and Posalasky (1964). In the case of control, H_2O_2 was omitted from the incubation medium.

Measurements used in the text are taken using either an ocular micrometer or a square ocularmicrometer.

Photographs were taken using a Carl Zeiss photomicroscope.

In grouping the gymnosperm species, Pant's (1957) classification is followed.

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CHAPTER IV

OBSERVATIONS

4.1 STRUCTURE OF THE SHOOT APEX

The shoot apical organisation is of some value in broad phylogenic studies as we see that a single cell initiator seen in the ferns gives way to establish an array of initiators that exhibit one or more stratified outer layers, with predominant anticlinal divisions (angiosperms). With a view to trace the evolutionary sequence, a group which deserved scarce attention earlier, has been selected. Members of different families and orders have been selected for study. Foster's (1938) theory of cytohistological zonation seems to be more useful than others in interpreting them and has been applied here.

4.1.1 CYCAS CIRCINALIS*

Terminal buds of plants aged more than ten years have been analysed in this study. The young bud is covered by brown, spirally arranged foliar primordia. The leaves are quite long and extend to a length of 1-3 metres. The

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*Publication of a paper entitled 'Shoot apical organisation and leaf histogenesis in Cycas circinalis' encomposing these results, is awaited soon in New Botanist.

petiole is long and spiny and carries 80-100 pairs of leaflets on the main rachis and the distal leaflets are incurled.

When viewed from above, the shoot apex is dome shaped and the height above the youngest leaf primordium varies from 8-300 μ and the diameter from 476-1115 μ. The dimensions of the apices vary with the plastochron. Four zones could be distinguished in the shoot apex, viz., apical initials, central mother cells zone, peripheral zone, and the pith rib meristem (Fig. 1, Plate VII).

Zone of apical initials

The surface cells, about 6-8 rows deep from the outer boundary of the dome-shaped apex constitute this zone. These cells do not form a tunica, as they are not arranged in well defined layers due to the frequent periclines (Fig.1, Plate VII). Both periclinal and anticlinal divisions occur in this zone, thereby contributing directly to the peripheral zone and the central mother cells zone. Thus, the apical initials are the ultimate source of all the cells of the shoot.

Zone of central mother cells

This zone, situated just below the apical initials, is globose or elliptical and the constituent cells are comparatively large, more vacuolated less chromophilic and with irregularly thickened cell walls. Divisions in various

planes occur in this zone. Groups of compactly arranged cells are seen here, the plane of divisions showing their origin from the same initial cells (Fig. 2, Plate VII). Schüepp's concept (1926) of 'massiges meristem' can be applied to this zone, which is more prominent than that of Cycas revoluta reported by Foster (1940). A junction belt of cells arranged transversely just below the central mother cells zone marks its boundary from the lower rib meristem. However, this is not like the cambium-like zone reported by Foster (1938) in Ginkgo biloba (Fig. 2, Plate VII).

Peripheral zone

This clearly demarcated zone has smaller but deeply chromophilic cells. It diverges from the surface initials on the flanks. Cells of this zone arise from the central mother cells zone and the surface initials on the flanks. As the flank meristem is traced down, a linear distinction is noticed between the inner and outer layers. Its outermost layers contribute to foliar development. The provascular tissues arise from the inner layers and are seen external to the central pith region, and as they are traced acropetally, disappear just below the peripheral zone.

Pith rib meristem

This zone arises from the central mother cells zone and the cells are more vacuolated than those of the peripheral zone. Anticlinal divisions predominate, giving rise to the pith proximally though periclinal and anticlinal

divisions are also noticed. The pith cells do not experience much elongation, but are responsible for the increase in girth.

Distribution of polysaccharides

Distribution of insoluble sugars in the different zones was qualitatively determined using histochemical methods. The periodic acid - Schiff's (PAS) reaction gave very good results. The polysaccharides stained intense purple red. The nuclei stained lightly and the cytoplasm lighter still (Fig. 4, Plate VII). The water soluble sugars could not be localised as they get dissolved by the repeated washing in water.

The deposition of the sugars is found to be minimum in the central mother cells zone (Fig. 3, Plate VII). The cyto-histological isolation of the central mother cells zone is clearer in sections treated for the polysaccharides. The accumulation of polysaccharides is highest in the rib meristem region. The abaxial side of the young primordia shows greater amount of carbohydrates than the adaxial one.

4.1.2 PINUS ROXBURGHII (Sarg.), PINUS WALLICHIANA (A.B. Jacks), PINUS INSULARIS (Endl.), PINUS GERARDIANA (Wall), PINUS MASSONIANA (Lamp) and PINUS PSEUDOSTROBUS

Of these, the first four are species native to India and the last two are introduced. The shoot apices of these species show a fundamentally similar configuration. Therefore a general description is given for all these and

differences are dealt with wherever they are observed. Only the external morphology and embryology of two plants, viz., P. roxburghii and P. wallichiana have been studied earlier but their shoot apical organisation have not been reported.

P. roxburghii, P. gerardiana, P. insularis and P. massoniana belong to the 2-3 needled hard pines while P. wallichiana and P. pseudostrobus are 5 needled soft pines.

Shoot tips were collected during the period of bud expansion. Rapid formation of cataphyll primordia associated with marked increase in height was quite pronounced in P. roxburghii than others. The shoot apex is a dome or a cone in shape with variations in diameter and height from species to species. 25-35 apices of each were examined and the mean values are given below.

Species	Height	Diameter
P. roxburghii	218	403
P. wallichiana	130	381
P. gerardiana	160	250
P. insularis	205	272
P. pseudostrobus	218	403
P. massoniana	118	289

All the six species show the same basic zonation, but vary in their distinctness. Four cytohistological zones are discernible: (1) apical initials, (2) central mother cells, (3) flanking zone, and (4) rib meristem. The zonation is

quite distinct in P. pseudostrobus and P. massoniana but not so sharp in P. roxburghii, P. gerardiana and P. wallichiana. The least sharpness is noticed in P. insularis (Plates VIII and IX).

Zone of apical initials

This is the most distal zone of cells. It is characterized by cells with big nuclei and less chromaticity. Divisions in both planes occur, pericliny contributing to the central mother cells and anticline to the outermost layer of flank meristem. Hence these cells may be considered as the ultimate source of all the cells of the shoot.

Zone of central mother cells

This zone occupies a position just below the surface layer at the summit of the apical dome. It is normally of 5-12 cells as seen in a longitudinal section. The zone is very well marked in P. massoniana and P. gerardiana (Plate IX, Figs. 3 and 4). Its cells divide in all the planes resulting in an irregular mass. The cells stain less intensely and show corner thickenings. They appear to be the biggest cells in the apical dome. The zone is augmented by the inner derivatives of the periclines in the surface layer at the summit and by divisions of its own cells. Cell divisions on the flanks of this zone contribute to the flanking zone, and those at the base are usually perpendicular to the long axis and contribute to the pith rib

meristem. Vacuolation of the cells of this zone seems to be maximum in P. roxburghii. In P. roxburghii and P. massoniana the nuclei of the central mother cells zone are double the size of those of pith rib meristem cells, while in P. wallichiana, the nuclei of central mother cells are slightly bigger than those of the adjacent cells.

Flanking zone

The flanking zone forms a cylinder of elongating and dividing cells around the zones of central mother cells and rib meristem. It is composed usually of 3-7 layers of cells with high frequency of mitosis. The number of cell layers increases proximally due to the periclines followed by anticlinal divisions (the 'T' divisions of Schüepp, 1917). The cells of the surface layer and the inner cell layers of the flank meristem behave similarly. Hence they are not described separately, as has been done in the case of P. canariensis (Pillai and Pillai, 1974). The cells stain deeply and the nuclei are comparatively smaller. The cells of this zone originate either by anticlinal divisions of the apical initials or by the contributions from the central mother cells zone or both. But no demarcations due to the dual origin can be made out in the tissue. But in some apices the inner layers of the zone that originate from the flanks of central mother cells zone have a somewhat stratified appearance towards the distal part of the apex due to the predominantly anticlinal divisions. The leaf or cataphyll primordia arise from the flank meristem by

division of the mother cells. Provascular elements, leaf and cortex arise from the same zone.

Pith rib meristem

This originates from the basal derivatives of the central mother cells zone, and displays a more or less regular arrangement of vertical files of cells which mature into the pith. These vertical rows of cells are a result of repeated transverse divisions. By means of the frequent longitudinal divisions followed by transverse septation of the resultant daughter cells the width of the rib meristem increases proximally.

The maturation of the pith cells from the rib meristem is evinced by the enlargement of cells and a high degree of vacuolation. The conical shape of the apex of P. roxburghii, P. insularis, and P. pseudostrobus is due to the regularity with which the rib cells are arranged in vertical rows. In P. insularis intercellular spaces are seen in the pith very close to the summit of the dome. The pith cells are either compact as in P. wallichiana and P. roxburghii or with intercellular spaces as in P. insularis. (Plate VIII)

The presence of resin cells and ducts is a common feature of all the species. The resin duct is formed of one or two layers of secretory cells. Though the usual resin cells arise distal to the pith rib meristem, it is seen in some of the cells of central mother cells in P. roxburghii.

In P. wallichiana, P. roxburghii and P. massoniana, the pith cells are mostly filled with a colored content, probably resin; in others nearly half the total number of cells exhibit this. The resin cells are either isodiametric or elongated. The elongated cells occur around the vascular elements, usually of some 2200-3300 μ below the tip. The resin ducts and provascular tissues occur more or less at the same distance away from the tip, usually 440 μ in P. massoniana or 550 μ in P. gerardiana. The number of elongated resin cells appear to be very few in P. insularis, P. wallichiana and P. gerardiana.

Except in P. pseudostrobus, all other apices exhibit the absence of sclerenchymatous cells. Crown is absent in all the members studied.

4.1.3 ABIES PINDROW (Spach)

Abies, the silver fir, includes 46 species and is represented in the western Himalayas by the Species Abies pindrow. It grows at a height of about 3050 metres interspaced with Picea smithiana, Pinus wallichiana and Taxus baccata.

The plant is easily distinguishable by the leaf scars left on the young twigs. The leaves at the apex are arranged spirally, but at lower levels show irregular arrangements.

Parke (1959) studied the growth periodicity of Abies concolor and noticed the shoot apex to have the maximum clarity of cytohistological zonation at the starting of growth phase I, i.e., during bud elongation. As the main point of study of A. pindrow was the shoot apical organisation, collections were made at the same growth phase. The buds show maturing foliage leaves and the initiation of cataphylls. The apices have attained 5-9 cms growth in the new season and were undergoing internodal growth.

Unlike other members of the conifers, the shoot apex is seen in a shallow cup, formed by the lateral outgrowth of the ground meristem, bearing all the scale primordia more or less at the same level as that of the shoot.

The apex is dome-shaped (Fig. 1, Plate X) with the height of the apex ranging from 88 μ to 154 μ and diameter from 220-385 μ. The average height and diameter of 30 apices are 128 μ and 275 μ respectively. The average diameter of the bud, at the level of the youngest leaf primordium is 3 mm. The cortex is somewhat spongy, due to the occurrence of big intercellular spaces, which may be filled with resin. This seems to be a speciality of this species.

The other boundary of the apex exhibits a surface layer, the cells of which at the distal end are more or less isodiametric, while those on the flanks are elongated in a plane perpendicular to the shoot axis (Fig. 1, Plate X).

The distal or apical cells are bigger, less chromophilic and with bigger nuclei, as compared to those of the rest of the surface layer. Hence, they can be given the status of apical initials. Periclinal divisions are rare, in the cells of the apical initials zone as compared with the rest of the surface layer.

Just below the apical initial zone, a few poorly stained but highly vacuolated cells constitute the central mother cells zone. They divide in all planes. The cells are slightly bigger than the apical initials, and are isodiametric. The deposition of cell wall materials, gives the impression of corner thickenings and the cell walls show more or less the same size, but differ greatly from those of nearby cells. The average diameter of the apical initial cells is 13.5 μ and that of a neighbouring cell is 7-10.8 μ. The origin of central mother cells zone can be traced back to the apical initials.

The lateral and basal derivatives of central mother cells zone constitute the main bulk of the cell population of the shoot apex. The lateral derivatives of the central mother cells constitute the flank meristem, while the basal derivatives formed by the continued periclinal divisions, develop into the pith rib meristem. The pith rib meristem cells constitute 4-5 vertical rows of thin-walled rectangular cells across the stem, which mature into pith cells proximally. They are usually noticeable 6-7 cells below the shoot tip. The cells of pith rib meristem enlarge and show

vacuolation progressively. Their further destiny could not be followed as almost all the cells are filled with coloured deposit (Figs. 1 and 2, Plate X). The nuclei of the cells of the pith rib meristem are comparatively smaller in relation to the total cell volume, than cells of the flanking zone except those in the proximity of the mother cell region.

The cells of the surface layer on the flanks, along with those cells, surrounding the pith mother cells, form the flank meristem. These cells show the maximum chromaticity and form a cylinder around the pith mother cells. This zone serves as the organogenic region of the apex. The provascular elements can be seen developing acropetally, a little below the tip.

Thus, the shoot apex of Abies pindrow can be said to have four cytohistological zones, viz., (1) apical initials, (2) central mother cells, (3) pith rib meristem, and (4) flank meristem.

4.1.4 TAXUS BACCATA

A reinvestigation of the shoot apex of Taxus has been attempted as only two attempts have been made in the family Taxaceae, since the emergence of modern concepts of apical organisation. Kemp (1943) studied the apical meristem and seasonal activity of Torreya buds and Singh (1961) of Cephalotaxus drupacea.

Taxus baccata, the yew, grows in the Himalayas along with Abies and Picea. The plant is distinguished from Abies pindrow by its small structure and decurrent growth habit.

The overwintering buds collected in the month of May showed extension growth. The domed apex of the shoot (Fig. 3, Plate X) is comparatively small, but with an average height of 106 μ and width of 218 μ with the height to diameter ratio of 1:2 (for 20 apices). The range of height and diameter fluctuated between 65-130 and 180-240 μ.

The pattern of cytohistological zonation with the 4 zones resembles that of Abies. Hence only a brief description is given.

Apical initials

3-4 highly staining large cells at the extremity of the shoot constitute this zone. The nuclei are large and the cell shows little vacuolation. The cells are elongated in a direction perpendicular to the long axis of the shoot due to the predominantly anticlinal divisions. Periclinal divisions of these cells are rarely seen.

Subapical initials

The basal derivatives of the apical initials enlarge slightly in size and constitute 4-6 subapical initials. The derivatives of these in turn, produce the body of the shoot apex. The cells are round, highly stained and large

nucleated. As irregular divisions occur in the cells of this zone, a globose shape is attained by this zone.

Peripheral zone

The derivatives of the flanks of the apical and subapical initials constitute the peripheral zone. The cells are small, heavily stained and highly cytoplasmic. The zone is 4-6 cells wide and the arrangement of cells is irregular. The cells of the surface layer show periclinal divisions at the time of primordial initiation. The surface layer on the flanks shows predominantly anticlinal divisions, which gives the appearance of a distinct surface layer.

Rib meristem

There is no interposition of pith mother cells beneath the subapical initials. Instead, at the base of the subapical initials, 4-6 vertical rows of highly vacuolated cells show less stainability than the surrounding cells. This is the pith rib meristem. Downwardly they merge with the pith, where the cells show more vacuolation and maturation. In the pith the central cells mature earlier than the nearby cells.

4.1.5 EPHEDRA FOLIATA

The present investigation further substantiates the observations of Deshpande and Bhatnagar (1961), Pillai and Pillai (1974) on its apical organisation. Details are given in another chapter that deals with its floral initiation.

4.2 GROWTH PERIODICITY AND STRUCTURE OF THE SHOOT APEX

4.2.1 PICEA SMITHIANA

With a view to study the seasonal changes occurring in the shoot apex of arborescent gymnosperms that grow in the temperate regions, two conifers from the western Himalayas viz., Picea smithiana of Abieteae and Cedrus deodara of Pineae were selected. Buds, about 3 cm long were collected monthly.

Cedrus and Picea exhibit seasonal changes in the activity as well as in the morphological features of the apices. Though there is no change in the basic cytohistological zones throughout the annual cycle, regularly occurring decrease in the distinctness of the zonation could be detected as was reported by Kemp (1943) in Torreya and Sterling (1946) in Pseudotsuga in the winter buds. But, the preservation of the size of the central mother cells zone as noticed by Sacher (1954) in Pinus ponderosa has not been observed here.

Shah and Thulasi (1967) reported new bud formation in Picea smithiana growing in Chakrata in Dehradun Forest Division in mid-April but did not study the periodic fluctuations. Shah and Thulasi (1967) recognised five zones in the apices of mature plants of Picea smithiana viz., (1) apical initials, (2) sub-apical initials, (3) flank meristem, (4) rib meristem, and (5) surface layer on the

flanks. The basic architecture of the apex found in the present study is the same as that reported by Shah and Thulasi (1967). The surface layer can be delineated from the inner cells by the predominantly anticlinal divisions in this layer and the constituent cells showing more depth than length in the actively growing shoot apex. But the view of Shah and Thulasi (1967) that pericliny occurs only during leaf formation does not conform with the present study (Fig. 2, Plate XI).

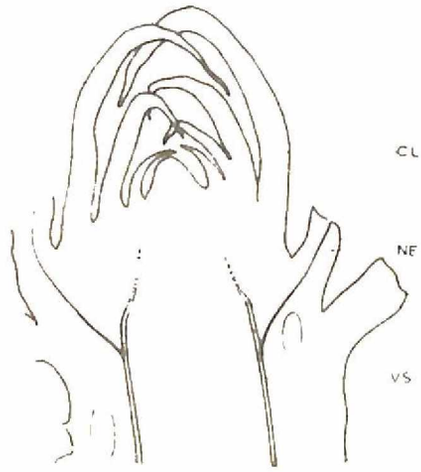
Here, for describing the bud in various seasons, the terminology used by Singh (1961) is followed. Four periods are noticed viz., (1) rest, (2) period of elongation, (3) period of cataphyll formation, and (4) period of foliar primordial development.

Resting bud

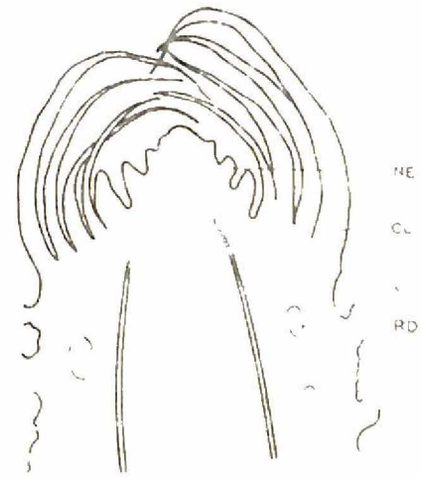
The period of inactivity or dormancy that starts in the month of November prolongs for nearly four months. In these dormant apices (dome-shaped) the leaf primordia are borne above the crown in an appressed form. There is leaf elongation during this time. The average height of the bud above the crown is 1300 µ. The pith cells are completely filled with some densely coloured contents that are absent in the region beneath the crown. The fully differentiated vascular elements as well as resin canals are traceable upto the region beneath the crown, while only some procambial elements which are not well-developed are observed above the

DORMANT BUD

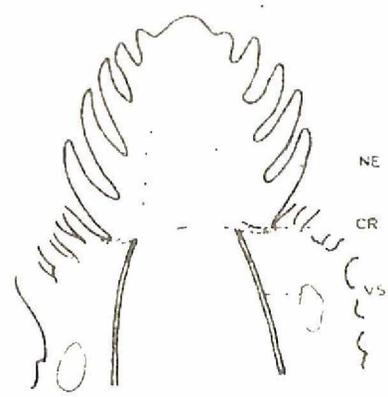
BUD EXPANSION



CATAPHYLL PRODUCTION



NEEDLE PRODUCTION



INITIATION OF CROWN

CL - CATAPHYLL
CR - CROWN
NE - NEEDLE
RD - RESIN DUCT
VS - VASCULAR SUPPLY

100μ

PLATE I DIAGRAMATIC REPRESENTATION OF THE GROWTH PERIODICITY OF VEGETATIVE BUDS OF PICEA SMITHIANA

crown. Few division stages, could be noticed in the dormant apex affirming the state of inactivity. However, relatively more number of divisions were seen in the leaf primordia.

The zonation is not sharp in the domed apex. The height to diameter ratio is 1:1.54 for average values of 134.6 μ to 206 μ for height and diameter respectively. The subapical cells are slightly bigger than those of the adjacent cells (Fig. 5, Plate XI). Pericliny in surface layer is noticed on the lower flanks of the apex (Fig. 5, Plate XI). Parke (1959) has diagramatically shown the resting bud of Abies concolor having part of the central mother cells below the level of the youngest leaf primordia. But in Picea the complete zone of central mother cells is above the level of the youngest leaf primordia.

The surface layer shows isodiametric cells with occasional pericliny at the lower slope on the flanks. The apical initials are very few, only 5 to 7 cells. The cytoplasm stains densely and the cells show vacuolation. The peripheral zone is composed of 4-5 vertical rows of cells and the rib meristem of 5 to 7 rows of cells. The surface layer does not merit the status of a tunica as periclines are noticed there.

Bud elongation

After the cold February days, the bud starts elongation. The leaves show sign of maturation by the development of

vascular tissues. The vascular elements which were below the crown in winter develop above it and show progressive acropetal development into the leaves. By the middle of elongation phase in April, differentiation of the procambium is observed 650 μ below the tip of the shoot. By April-May new buds develop from the leaf axils, a little below the tip. Internodal elongation continues till the end of June. The bud increases in height considerably and the pith cells show the maximum elongation. The insoluble polysaccharides are not observed in these months (Fig. 2, Plate XIII). Obviously they are being utilized during the renewed activity.

With the spring burst of the bud in March, the apex shows increase in size and thus shows similarity with the buds of Cephalotaxus (Singh, 1961). But the reverse situation is reported in Podocarpus gracilior (Pillai, 1963b) and Torreya (Kemp, 1943). Evidence for the fact that the surface layer is quite active is provided by the two telophases of Fig. 1, Plate XII. The cell walls are thin comparatively. The number of subapical initials and pith mother cells increases (Fig. 1, Plate XI). The extent of the pith rib meristem is found to have increased.

The elongation of the overwintering bud is not only due to cell elongation but also due to cell division. The pith cells above the crown undergo cell division. Cell multiplication that occurs in the crown region shows the long axis of the spindle figure oriented parallel to the long axis of the shoot. The maturation of the pith cells takes place only after elongation.

The elongation of the pith cells is as much as 195 μ, for a cell placed 5500 μ below the tip from the original size of 30 μ. The resin containing cells are very few and no resin duct is noticed in the initial months of elongation, but later develop acropetally. By March, xylem differentiates, followed by differentiation of stone cells in the pith, but not in the cortex.

Period of cataphyll formation

During elongation, the apex shows organogenic activities such as the formation of appendages. New bud formation is noticed from March to April and cataphyll formation from May to August. Shoot elongation and scale formation continue concomitantly for sometime.

The apex changes from a dome to a cone with the initiation of cataphylls. The height diameter ratio of 1:1.46 of the apex of the elongating bud increases to 1:1.5 with the production of cataphyll. The zonation is clear and the cells show dense staining (Fig. 2, Plate XI). The apical initials and the subapical initials are bigger than the nearby cells. The rectangular cells of the surface layer on the flanks are arranged side by side with their planes of division perpendicular to the long axis of the plant, compared to the more or less isodiametric cells of this layer in the dormant bud. In Fig. 1, Plate XII, a surface cell shows periclinal division during cataphyll production. Two to three nucleoli are usually observed. In Torreya, Kemp (1943) finds no division on the flanks. The flank meristem in Picea at this stage is

5 to 7 cells deep and the pith rib meristem 6-7 rows of cells across. The origin of the resin ducts and differentiation of the vascular elements are noticed at the same height, 275-550 μ below the apex.

Period of foliage leaf production

During the months of July and August the apex attains the maximum size with the primordia developing into leaves rather than to cataphylls.* During the four months starting from August through November profuse production of leaf primordia is noticed. The apical bud retains an average height of 124 μ and width of 248.6 μ for the apex during this time. The apex appears like an inverted cup (Fig. 3, Plate XI). The zonation is clear. The cells show less vacuolation. The nuclei are big (Fig. 3, Plate XII). The surface layer shows periclinal divisions at the summit as well as on the flanks. Resin cells are seen reaching the zone of subapical initials and most of the pith cells are filled with coloured substances. At times the subapical initials are partly below

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*The cataphyll and leaf primordia are difficult to be distinguished in the initial stages. But they can be detected by counting the number of primordia on the flanks. There is only one pair of newly initiated primordia during cataphyll production while more pairs can be seen burgeoning from the flanks during foliage leaf production. As the development proceeds, they can be distinguished from each other. The cataphyll undergoes rapid marginal expansion while foliar primordia first increase in radial thickness. In the foliar primordia the longitudinally elongated procambial cells appear soon. Moreover the mesophyll is poorly developed in cataphyll.

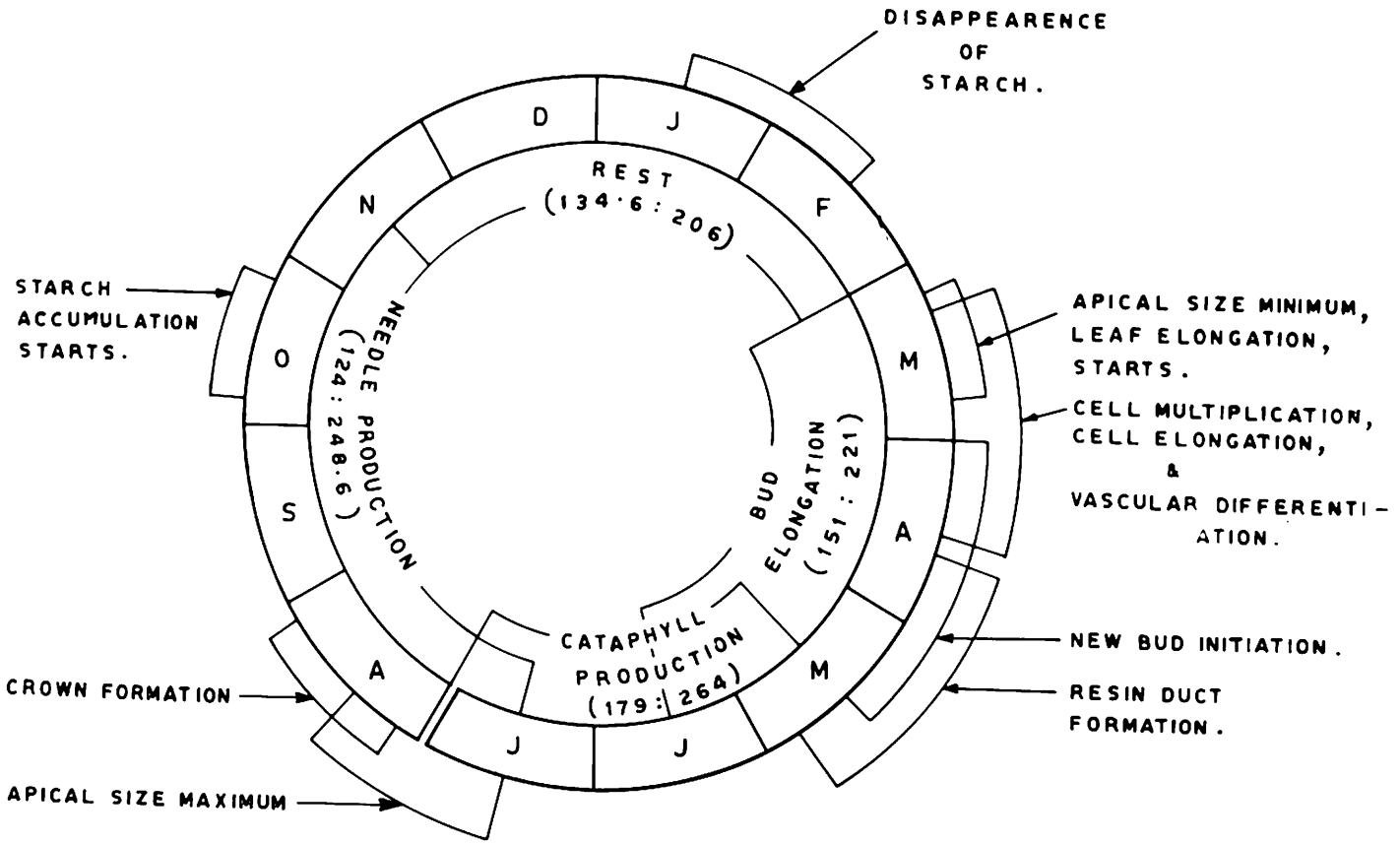


PLATE.2 MORPHOGENIC CYCLE OF VEGETATIVE TERMINAL BUDS OF PICEA SMITHIANA GROWING IN SIMLA, BASED ON MONTHLY COLLECTIONS MADE DURING 1972 — 73

the level of the youngest leaf primordia unlike those reported by Parke (1959) in Abies concolor (Fig. 4, Plate XI). By November about 9 pairs of primordia are seen flanking the bud. These primordia appressed closely to the central cylinder of the shoot, constitute a telescoped bud or rest bud (Fig. 1, Plate XVII).

In Picea the accumulation of starch in the bud starts in the month of October and maximum storage is noticed in January (Fig. 1, Plate XIII). Then onwards the amount declines. The granules are seen spreading upto the lower portion of the subapical initials zone. The granular size of sugars present in the pith as well as cortex increases more and more distally. From March onwards the apices are devoid of polysaccharides except those constituting the cell wall (Figs. 2 and 3, Plate XIII).

The protein distribution seems to be uniform in all the months (Figs. 5 and 6, Plate XI). The peripheral zone exhibits denser staining while the pith region shows the least. The histone staining is answered weakly in the cytoplasm and strongly in the nucleus. It is seen that there is no variation in histone concentration in the apices during different seasons, both the nucleus and cytoplasm stain uniformly (Figs. 4, 5 and 6, Plate XIII).

4.2.2 CEDRUS DEODARA

Cedrus is represented in India by the species C. deodara which yields the strongest conifer wood that can be equated with teak wood from the weight point of view. The plant is cultivated in the Himalayan forests, at an elevation of 1220-3050 metres and commonly occurs in the range of 1830-2590 metres. Of all the four species of Cedrus, C. deodara is the first one whose shoot apex has been studied throughout the year.

The plant is about 60 metres high with horizontal or slightly ascending or descending branches. Unlike Picea two types of branches are met with, one of unlimited growth or long shoots and the other of limited growth or dwarf or spur shoots. Needles are seen spirally arranged in the long shoot with 3/8 phyllotaxis, while in the dwarf shoot, a fascicle of needles in a pseudowhorl is seen. Leaves are acicular, rigid, sharply pointed and above 4 cms long. Scale leaves are usually devoid of resin ducts. A maximum of 8 resin ducts is observed in the cortex of the shoot.

Long shoots grow from the terminal bud and from a few of the upper axillary buds, situated on long shoots of the previous year. Short shoots have a fascicle of leaves originating from the other axillary buds and exhibit the same basic features as the long shoots except the internodal elongation.

Structure of the shoot apex

The only report about the shoot apex organisation of Cedrus is that of Koch (1891) of Cedrus libani. He considered it to have an outer mantle of tissue covering an inner core of loosely arranged cells.

The architecture of the shoot is quite similar to that of Picea and the outer surface layer is discernible into two zones (Fig. 1, Plate XIV). Thus five zones are distinguishable (i) apical initials, (2) surface layer on the flanks, (3) subapical initials, (4) flank meristem, and (5) pith rib meristem.

The apical initials are distinguishable from the cells of the flanks by their larger size and weak staining. They occupy the summit of the apex in longisection and consist of 3-5 cells with big nuclei. The apical initials perpetuate themselves by their anticlinal divisions and add to the subapical initials by periclines. The subapical initials are quite distinct as a zone during the period of high activity. Though divisional phases have not been noticed, the occurrence of division is adduced by the newly formed thin cell walls. The subapical cells are as big as the apical initials in the active period, with some intercellular depositions at cell junctions. The division of the outer limiting layer cells is both periclinal and anticlinal, thus, ruling out the possibility of a tunica layer (Fig. 1, Plate XIV).

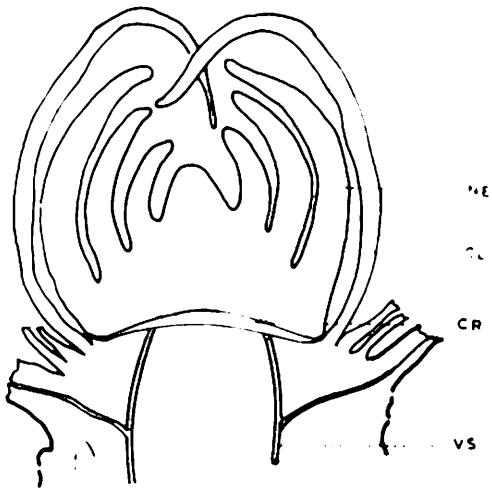
The organogenic region of the apex is composed of the surface layer on the flanks and the subjacent flank meristem.

It is 5 to 8 rows of cells deep. The cells stain deeply and are more or less of the same size. No stratification is noticed. These tissue layers occupy most of the total volume of the apex and surround the central region of the apex including the dome of apical initials. The origin of the zone can be traced to the subapical initials, the apical initials and surface layer on the flanks. The provascular elements show differentiation from the flank meristem at different levels during the different seasons. The surface layer on the flanks shows anticlinal as well as periclinal divisions.

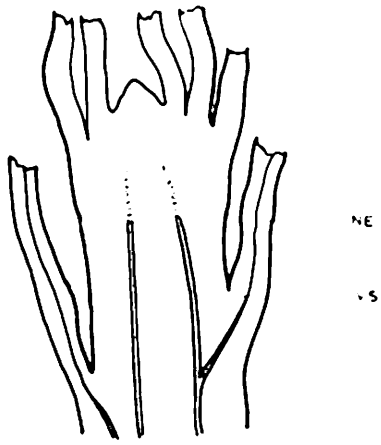
Cedrus exhibits pronounced seasonal activity like Picea but the fluctuation from one period to another is more than that of Picea. Four stages viz. (1) period of bud dormancy, (2) period of elongation, (3) period of cataphyll formation, and (4) period of foliar leaf production are recognised. The architecture of the meristem is quite similar in the different phases with slight variations.

Period of bud dormancy

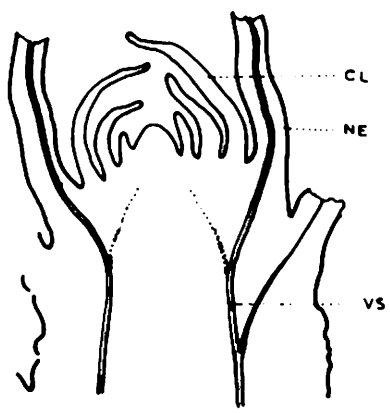
Unlike in Picea smithiana, the period of rest in Cedrus lasts for a longer time. The vegetative bud enters dormancy during the last week of September and the apex is in the inactive stage till the end of February. The apex at this stage is at the tip of a telescoped shoot, with an array of foliar primordia, encircled by the scale leaves. A few horizontally elongated cells form a plate in the pith which



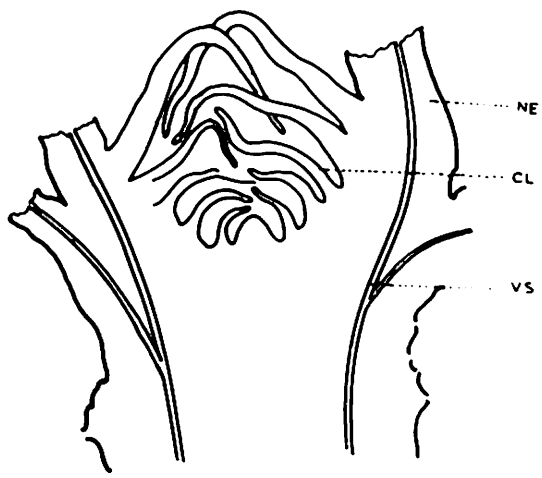
DORMANT BUD



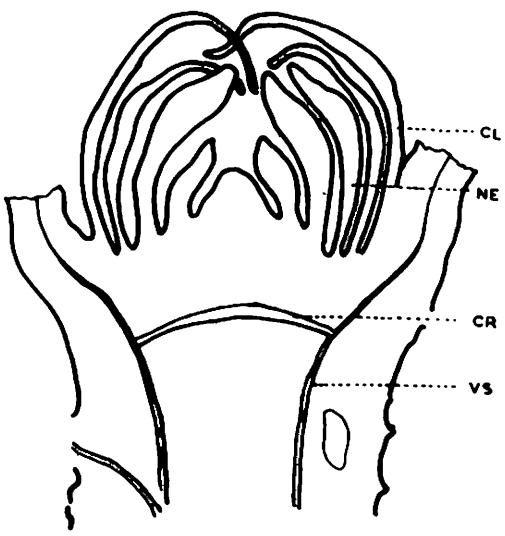
BUD EXPANSION



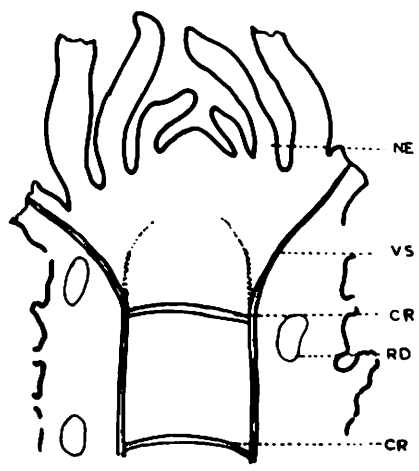
END OF PERIOD OF BUD EXPANSION



CATAPHYLL PRODUCTION



NEEDLE PRODUCTION



CL - CATAPHYLL
CR - CROWN
NE - NEEDLE
RD - RESIN DUCT
VS - VASCULAR SUPPLY
100M

ONE DWARF SHOOT - NEEDLE PRODUCTION

PLATE 3. DIAGRAMATIC REPRESENTATION OF THE GROWTH PERIODICITY OF VEGETATIVE BUDS OF CEDRUS DEODARA.

subterminally, form the crown (Fig. 2, Plate XIV). Even in the dormant bud a slight activity of the apex appears to occur, because the length above the crown increases with successive months. In September the crown is seen about 440 μ below the tip, in October 600 μ, January 660 μ and March 780 μ.

The apex is more a dome than a cone with steep slopes with a height to diameter ratio of 1:1. (Fig. 3, Plate XIV). The maximum volume of the apex is found to be in the dormant stage. No divisional phases were noticed in apices stained with Feulgen (Figs. 1 and 3, Plate XV). The cells of the surface layer are bigger than others. 3-5 distal ones constitute the apical initials. The cells of the surface layer are well vacuolated with round nuclei and show pericliny on the slopes. The nuclei of the apical initials are bigger and less densely stained than those of the outer cells of the surface layer. The zone of subapical initials shows 5-12 cells in the longitudinal section and has smaller nuclei than the apical initials. The flank meristem is located on the sides of the subapical initials. It does not produce any appendage during the dormant stage. There is elongation of the foliage which becomes maximum in March.

Period of elongation

Period of elongation of the dormant shoot lasts for nearly three months starting from the end of March till the last week of May or beginning of June. The leaf primordia emerge out of the mantle formed by the interwoven scale leaves

and expand into mature leaves (Fig. 4, Plate XIV). The vascular traces which were beneath the crown till April cross it by May, and undergo acropetal differentiation fast. In the April bud, the crown is 1456 μ below the tip.

The apex is conical now with an average size of 108 μ in height and 162 μ in diameter (Fig. 5, Plate XIV). The apical initials are big and less stained. The surface cells show periclinal as well as anticlinal divisions (Fig. 2, Plate XV). The subapical initials are quite big with prominent nuclei. Resinous material is observed reaching the pith mother cell zone but resin ducts are not seen. The average length of a pith cell located 1760 μ away from the tip in a dormant bud is 33 μ as against its counterpart 80 μ long in an elongated bud. With the approach of May, resin ducts appear. The resin canals originate during the process of elonation. Other features associated with bud elonation are differentiation of vascular elements and sclerenchyma and initiation of axillary buds.

Period of cataphyll formation

The period of bud elongation and the period of new bud formation cannot be separated in the case of Cedrus. Cataphyll production is first observed at the beginning of May and goes on till the end of July. An increase in the height and diameter of the apex is observed along with the cataphyll production in most cases. The continued production of cataphylls from the flanks makes the shoot apex to be ~~located~~

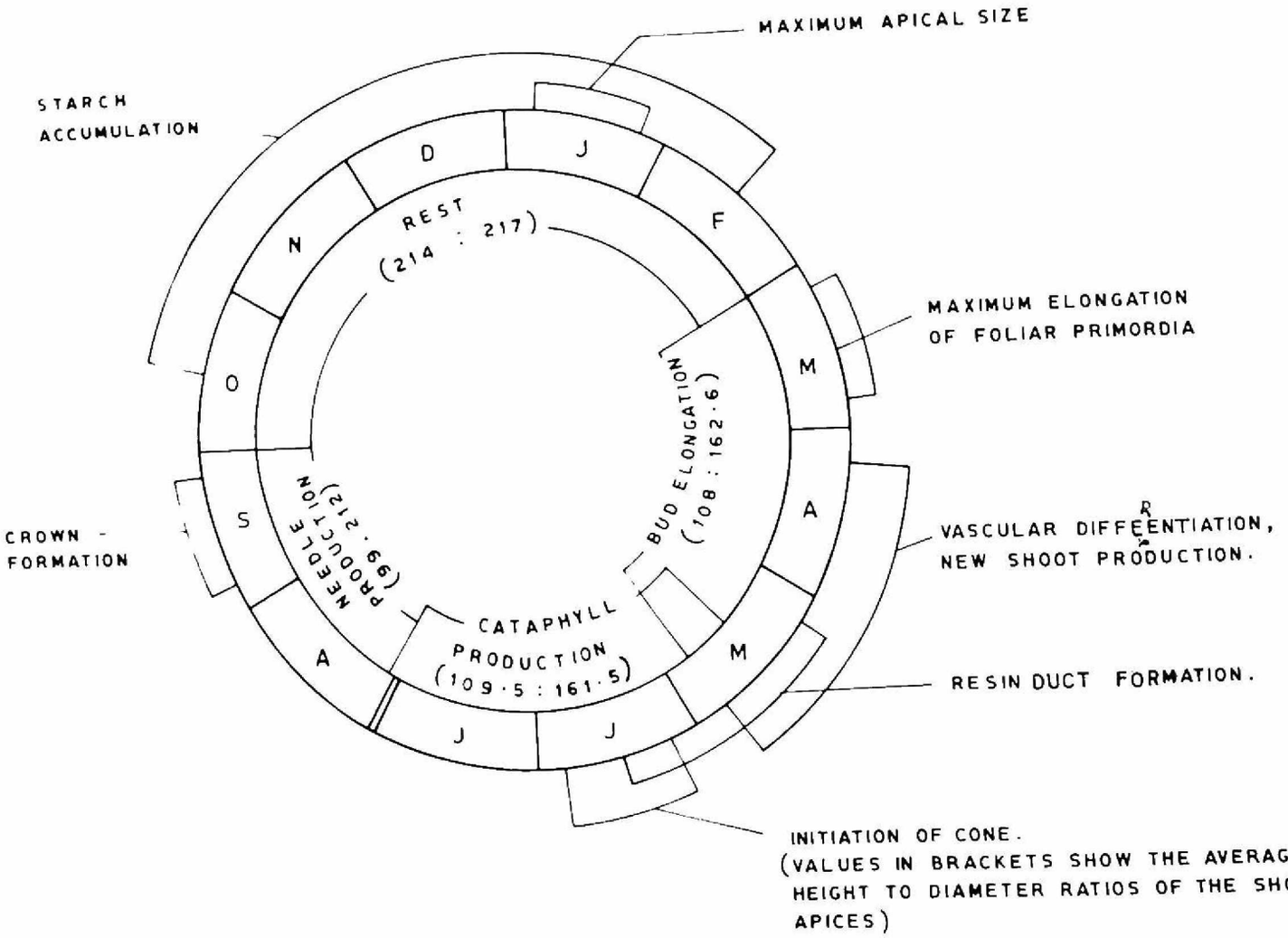


PLATE.4 MORPHOGENIC CYCLE OF VEGETATIVE LONG SHOOTS OF CEDRUS DEODARA GROWING IN SIMLA, BASED ON MONTHLY COLLECTIONS MADE DURING 1972 – 73.

located in a depression (Fig. 6, Plate XIV). The cells show less vacuolation and intense staining. The number of cells in the subapical zone and the pith rib meristem zone increases. Some of the dwarf shoots collected in June show their apices transforming into cones.

Period of foliar leaf production

Cataphyll formation is followed by the production of foliage leaf primordia. During the hot days of July and August leaf primordia are produced.

The apex is smaller with an average height of 99 μ and diameter of 212 μ and shows clear zonation. The cells are thin-walled and with less dense cytoplasm. In all the apices irrespective of the plastochron, the subapical initials zone is above the level of the youngest leaf primordia. The height/diameter ratio is the maximum at this stage being 1:2.1. Side by side with the production of leaf primordia, cells located at the base of the new bud at the junction of the new cataphylls and foliar primordia form the crown.

In Cedrus granules of polysaccharides appear in the bud in September (Fig. 1, Plate XVI) and are present in all the dormant buds. Maximum accumulation is noticed in January and February and then there is a decline. By April polysaccharides are completely absent from the portion above the crown (Fig.2, Plate XVI). Below the crown the granules continue to exist. The total protein and histone do not show variation in stainining density in apices of the different months (Figs.3-5,

Plate XVI). But variation is noticed from zone to zone of the apex.

In both Cedrus and Picea there are transverse divisions in the pith cells in the region above the crown at the time of internodal elongation. Along with the elongation, resin ducts also develop. Resin contents of pith cells move to the ducts which thereby regain their normal nuclear shape from the sickle shape caused by the accumulation of the resinous material.

4.3 CROWN FORMATION AND DORMANCY OF THE APICAL BUD

To a certain extent, the continuity of the pith in the terminal bud depends on the arrangement of leaves around the bud. In plants with whorled or opposite phyllotaxy the pith shows a discontinuity, due to the presence of nodes and internodes. As the node refers to the part of the stem at which one or more leaves are attached, it should exist even in plants with spiral and alternate phyllotaxy also. But in the latter case the discontinuity becomes only of theoretical interest because structural differences are not easily noticed in a young bud sectioned longitudinally.

Even from early nineteenth century, there are reports of the discontinuity of the pith in plants with spiral phyllotaxy, but not of regular occurrence. Hartig (1839) and Schacht (1853) noticed structures morphologically similar to the nodes, but different from them in details. Various names

were coined to distinguish such structures but few details were given.

A precise description of such a structure was first given by Busse (1893) based on his studies of some conifers including Abies alba. He used the term "Knospenscheide" to refer to the plate of tissue that interrupted the boundary of the growth of every year in the bud. The structure was rediscovered by Lewis and Dowding (1924). They gave the name "crown". They conducted some dye penetration experiments, in the light of which they ascertained its role to be related to dormancy. Later, Korody (1937) described the same structure in Abies concolor and Picea excelsa but preferred the term "Kollenchyma plate".

More recently, the existence of the crown has been reported in Abies concolor (Parke, 1959), Pseudotsuga taxifolia (Sterling, 1946), Sequoia sempervirens (Sterling, 1945a), Torreya californica (Kemp, 1943), Larix decidua (Frampton, 1960), Cephalotaxus drupacea (Singh, 1961), Hibiscus syriacus (Tolbert, 1961) and Podocarpus (Pillai, 1963a).

The Norwegian forest scientist, Venn (1965) studied some anatomical features of the crown in Picea abies and some other conifers. He prefers the term "nodal diaphragm" to the crown. Romberger (1963, 1966) stresses the need of probing further into the existence of a crown as he doubts the role of the crown to be associated with dormancy. In one of his

recent papers, Romberger (1975) puts, "The nature of the secondary wall materials, their origin, and the possible role of the crown in controlling bud dormancy have never been studied in detail".

Except these stray reports of its occurrence and structure, no detailed study has been made regarding its development and possible function. An effort in this line is attempted here.

Among all the gymnosperms studied here, two conifers, Picea smithiana and Cedrus deodara are observed to possess the crown. Studies of monthly collections of the materials enabled its development to be traced.

Structure

Dormant buds of both the species exhibit the crown, subterminally making a boundary between the young and old portions of the stem (Figs. 1 and 2, Plate XVII). It consists of a horizontal plate in the pith and cortex formed of 3-6 rows of cells and appears like a girdle. The pith below the crown is a little widened and a cavity may develop. The cells constituting the crown are isodiametric, more or less uniform in size and show highly thick walls. The nuclei are distinct and show intense staining with Feulgen (Fig. 1, Plate XVIII). The cells show vacuolation and protein bodies are noticed for one year. Some of the cells bear granules of polysaccharides and resinous deposits (Fig. 3, Plate XVII).

The cells differ from sclerenchyma and collenchyma in their wall constitution. The cell walls show high affinity for periodic acid Schiff's reagent indicating the greater accumulation of polysaccharides. Phloroglucinol and Ruthenium red tests are answered positively indicating lignin and pectin in the cell wall constitution. The sections tested for lignin show its presence by red coloration, only to the crown cells and xylem elements (Fig. 5, Plate XVII).

Development

As in structure, in development too there is close relationship between the crown of Picea and Cedrus. It starts its development during August-September probably with the advent of autumn. By this time the cataphyll production has stopped and foliar primordia are being initiated.

The first change towards the development of the crown is the alignment of some pith cells in a particular fashion, in the form of a girdle, showing close divisions evidenced by the shape of the cells (Fig. 3, Plate XVIII). The occurrence of the crown is at the junction between the region of initiation of foliar primordia after cataphyll production.

In Cedrus this subterminal structure originates about 450 μ below the tip and in Picea about 650 μ. The cells in this region are 6-7 layers thick and are horizontally elongated. Nuclei are active and similar to those of nearby pith cells (Fig. 1, Plate XVIII). Histone- and proteins-tests are answered positively in the nuclei and cytoplasm.

By October, starch accumulates in the bud, slightly in the crown, and intensely in the regions above and below the crown. In Cedrus sclereids differentiate below the crown in the cortex. The height above the crown averages 550 μ in in Cedrus and 880 μ in Picea. In Picea some coloured contents accumulate in some of the crown cells and the nuclei of those cells are deformed and crescent-shaped. The material collected in November indicated greater thickness of walls of 4-8 cell layers and in Picea, the cell walls for the first time answer the lignin test (Fig. 4, Plate XVIII).

By December-January when the apex has entered into the dormancy phase, the portion above the crown measures about 1760 μ in Picea and 780 μ in Cedrus. Starch has accumulated in the crown cells. The protein bodies are retained in the cytoplasm. The cell walls show further thickening and exhibit simple pits. The process of cell wall thickening proceeds in a special fashion. Initially the corners of the intercellular spaces among the crown cells thicken and subsequently all around the cells. This pattern of thickening recalls that of collenchymatous cells which might have been the reason for some of the earlier workers to call it a kollenchyma plate (Korody, 1937).

The dormant bud shows the vascular traces reaching just below the crown. No resin duct is noticed in the region above the crown. The pith cells above the crown are filled with some colored contents sparsely in Cedrus and more or less completely in Picea.

With the approach of spring, internodal elongation takes place and the vascular traces develop above the crown (Fig. 6, Plate XVIII). All the cells above the crown contribute to the elongation of the bud but none below the plate. Resin ducts are also seen to develop in the region above the crown. The starch grains that were sparsely dispersed in the crown cells disappear.

With internodal expansion, the apex above the crown measures several cms. The apical activity is accelerated and the production of foliar primordia follows the production of cataphylls. The bud region above the crown experiences not only cell elongation, but multiplication too (Fig. 5, Plate XVIII). Division phases are met with in the region just above the crown. By next autumn another crown develops and thus one year's growth of the apex is recorded. Only one crown is formed in an year.

A two year old crown shows (Fig. 5, Plate XVII) very compactly arranged cells with highly thickened walls. But by this time the thickened cells of the cortex become separated from their counterpart in the pith due to the development of the vascular elements. As a result in such an old bud, the crown is clear only in the pith region.

Even when the nuclei of the pith cells above and below the crown degenerate the crown cells show well organised nuclei (Fig. 4, Plate XVII). But in the course of time the amount of

feulgen-positive material inside the cell show sparse distribution and later disappearance.

4.4 INTERCALARY GROWTH IN EPHEDRA FOLIATA

The primary growth pattern of the aerial system of Ephedrales is different from those of the Coniferales. The Conifers may exhibit long and dwarf shoots that are absent in Ephedrales. The aerial body of Ephedrales is composed of a succession of nodes and internodes in their external morphology.

The node*, used here, refers to the region wherefrom the leaves originate. For Conifer buds, this is of only theoretical existence as it is not recognisable even under the microscope. But in Ephedra, nodes and internodes are quite distinct, and the distal internodes from the apical meristem undergo more and more elongation. Thus the internodes play a key role in the primary growth of the plant.

Studies were made on Ephedra foliata to determine whether the internodal growth is due either to the intercalary meristem, similar to that present in grasses, or to the resumptive meristem, that shows a latent activity of the apical meristem as is reported to occur in Arachis hypogea gynophore.

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*In Forestry, the term node is also used for the region of attachment of long shoots or branch whorls. Thus two terms, uninodal and multinodal buds are used. The former stands for buds with one series of sterile and fertile scales with one expandable internode while the latter for buds with more than one series of sterile and fertile scales with several internodes (Kozlowski, 1971).

According to Esau (1965) the intercalary meristem is one that is inserted between more or less differentiated tissue regions. It differs from apical and lateral meristems by an eventual transformation into mature tissues. Its presence has been noticed in pine needles, stems of monocots and dicots (Cutter, 1971), mosses (French and Paolillo, 1975) and Pteridophytes (Golub and Wetmore, 1948). Except for the attempts of Bucholtz (1920) on Oryza sativa, Dayes-Dujeu (1957) on Ephedra monostachya, Evans (1965) on Eleocharis acuta, Fischer (1970) on Cyperus alternifolia, French and Paolillo (1975) on the seta of Funaria, Golub and Wetmore (1948) on Equisetum arvense, Jacobs (1947) on the gynosphore of Arachis hypogea, Kaufman (1959) on Oryza sativa, no extensive work has been done on the intercalary meristem.

The first series of leaves of the young plant is visible with the emergence of the cotyledons out of the seed coat. Within a period of one month, the internodal region, undergoes extension. The rate of extension of the different regions of the same internode was roughly observed by marking it into several equal segments using India ink and the positions of the markings measured after a period of one month. It was seen that the growth was more in the lower segment.

As this greater rate of growth in the lower portion can be either due to cell multiplication or cell elongation, further studies were made to determine which of the two occurs. The terminal apices of profusely growing vegetative branches were fixed and longitudinal sections were Feulgen stained.

These apices have several nodes that are in different phases of maturation.

Mitotic figures are noticed in the internodal regions upto 7th node, of which the 3rd and 4th internodes exhibited the maximum numbers. In the 3rd and 4th internodes, as divisional figures are randomly distributed, it is difficult to conceive whether cell multiplication is due to rib meristem activity of the shoot apex or to an intercalary meristem.

Chi-square, a nonparametric test, was applied to see how the phenomenon of cell multiplication at different regions of the internode fits within the framework of statistics. Chi-square at different segments was determined using the hypothesis that the mitotic index in any segment is equal to that of any other segment. In other words, the hypothesis says that a homogeneity with regard to cell multiplication is displayed all along the length of the internode.

This is explained by quoting a representative sample. The 4th internode of a profusely growing branch fixed in the early morning measuring 990 μ in length was divided into 11 segments each of 90 μ. The median longitudinal Feulgen stained section shows altogether 2004 nuclei of which 34 are in divisional phase, others in nondivisional phase (Fig. 1, Plate XIX). In each segment the number of divisional and nondivisional cells are counted and mapped, using a square ocular micrometer net.

Sl.No.	Observed divisional nuclei	Observed nondivisional nuclei	Total
1	0	122	122
2	2	149	151
3	0	151	151
4	2	170	172
5	2	188	190
6	6	195	201
7	3	190	193
8	4	205	209
9	4	161	165
10	7	214	221
11	4	225	229
	34	1970	2004

As some of the observed values of divisional figures fall below 5, they are merged with the neighbouring rows. Thus, the first 5 rows are merged together and rest in two each to give altogether four groups. Thus we get:

Observed divisional nuclei	Observed nondivisional nuclei	Theoretical divisional nuclei	Theoretical nondivisional nuclei	Total
6	780	13.35	772.9	786
9	385	6.67	387.8	394
8	366	6.34	367.8	374
11	439	7.64	443.3	450
34	1970	34.00	1971.8	2004

The calculated value of Chi-square is obtained using the formula

$$X^2 = \sum \frac{(O - E)^2}{E}$$

As the calculated value comes to 6.782, which is significant at 10% level against the tabled value of Chi-square, 6.251 with df = 3; the hypothesis is rejected at this level.

Further tests were carried out to find out, due to which group the differences occur. Again Chi-square is applied taking one group versus another using the formula

$$X^2 = \frac{N((ad-bc) - \frac{N}{2})^2}{(k)(l)(m)(n)}$$

Among the six sets, one set constituting the uppermost and lowermost rows, gives a highly significant value of 4.787 against the tabled value of 3.841 at 5% level with df = 1.

As statistically significant values are obtained for different internodes examined, it is concluded that the heterogeneity has come in due to the uppermost and lowermost regions, of which the former exhibits the least number of divisions and the latter, the maximum. As the high mitotic activity is not due to the rib meristem (in that case lower regions of internode exhibit lesser number of divisions than the upper regions) an intercalary meristem is active in the lowermost segments.

Internodal growth is also the resultant of cell elongation. The cells that are isodiametric with a diameter of 22 μ in the 3rd internode show manifold elongation in the older internodes. From 4th node downwards, the pith cells experience the maximum elongation and epidermal cells the minimum. The cells adjacent to the node exhibit comparatively lesser elongation. The average length of the central pith cells comes to 55 μ in the 5th internode, 88 μ in the 6th internode and 105 μ in the 7th. The central region between the 8th and 9th nodes exhibits an average cell length of 165 μ while the same in the 9th internode is 240 μ. The central pith cells in the 10th internode show an average length of 320 μ.

Thus, each cell expanding to its maximum length adds approximately 300 μ to the longitudinal axis of the plant. The maximum length noticed is 473 μ. No further elongation occurs below the 10th node. It is important to note that from this point downwards the xylem tracheids with double bordered pits are seen. The xylem elements from the 10th node upwards

are annular and spiral ones which can adjust to the process of elongation. From the 10th node downwards the internodal elongation is stabilized with the occurrence of other types of tracheids too.

Unlike other plants like, Arachis (Jacobs, 1947), in Ephedra the region of maximum activity and maximum elongation are not the same. Moreover, the nuclei of mature pith cells are round compared to the serpentine or elongated ones of the grasses. The epidermal cells at the distal node show elongated nuclei exhibiting a maximum length of 50 μ.

The intercalary meristem that is ill-defined in the young internodes (Fig. 2, Plate XIX) becomes quite sharp with maturation of the internode. Its identity becomes clearly noticeable from the 8th node onwards (Fig. 3, Plate XIX). It is seen as a plate formed of 3-4 rows of cells that are horizontally elongated, situated 7-8 cells above the lower nodal plexus. The distinction of the plate is made clearly in the pith as their cells are thin-walled, less vacuolated and horizontally elongated (Fig. 4, Plate XIX).

Before the development of the vascular elements, the plate is clear in the cortex. But later it becomes obscure in the cortex but not in the pith (Fig. 5, Plate XIX).

An approximately four year old stem bears the plate that has retained its identity (Fig. 6, Plate XIX). The component cells are thin-walled and nucleate. The pith cells above and below are anucleate and thick-walled with abundant pitting. Further, the pith cells show positive reactions to lignification test. The same figure shows the vascular elements passing above the plate touching its sides. Near the points

of contact with the vascular elements, the plate shows a greater number of cells. The cells retain their characteristics of having thin walls and dense cytoplasm.

It is seen that the living cells of the plate join with the ray parenchyma cells of xylem at its two extremities and thus establish a connection with the cortex.

In still older stems, the plate takes the shape of an inverted dome, but still retains its structural features. The size of the cells does not change as the plant grows older, as is usual with other cells. But in a ten or more year old stem some of the central cells of the plate develop pits and the nuclei degenerate. Still the band is clear and this distinction helps one to distinguish it even in a very old stem.

4.5 APICAL MERISTEMSAND VASCULAR DIFFERENTIATION OF PICEA SMITHIANA DURING SEED GERMINATION*

Apical meristems of Picea smithiana during germination were studied, starting from dormant embryo and about 0, 10, 25, 34, 45 and 90 days after soaking the seed in water (Fig. 1, Plate XX).

Shoot apex

The shoot apex of the mature embryo (dormant seed) is dome-shaped initially which changes into a cone after soaking in water (Fig. 2, Plate XX). The average height and width

*Published as a paper entitled "Anatomy of the mature embryo and seedling of Picea smithiana (Wall) Boiss. Proc. Ind. Aca. Sci. 81 B(3), 101-110, 1975.

of the shoot apex of mature embryo, are 91 μ and 200 μ respectively. The apex shows increase in size with advancement of growth and a 34 day old seedling shows an average height of 139 μ and width of 250 μ. The size of the apex does not change upto three months.

The zonation is vaguely distinct in the shoot apex of the embryo, partially due to the accumulation of granular contents. The zonation becomes clear after one month of germination (Fig. 3, Plate XX) and continues to be distinct during further development (Fig. 4, Plate XX). Five zones are distinguishable as in the case of apices of mature plants. As the cellular details of each zone is much the same as those of the mature plant a detailed account is avoided.

Root apex

The structure of the root apex in both the mature embryo and seedling are fundamentally the same (Figs. 5,6, Plate XX). 5-6 initials in the form of a disc are distinct in the mature embryo which form a transverse plate of 4 initials in the seedling root. These initials produce the stele and pith proximally, and columella distally (Figs. 6 and 7, Plate V). The radicular stele is broader than the stele of the seedling root, evidently due to frequent Körper divisions (Figs. 5,6, Plate XX). The deeply stained narrow cells of the stele in the radicle of the mature embryo can be easily distinguished from those of cortex. The colorless cells of endodermis makes the boundary between the stele and cortex distinct.

In the radicle of the embryo, the central cells of the stele mature into the pith a short distance proximal to the initials while in the seedling root apices the pith does not mature so closely. In the seedling root apices the columella is uniformly 4 cells wide, while in the mature embryo, the cclumella is wider.

The cells around the initials cut off the cortex proximally and the cap distally. Kappe divisions occur in the pericolumnar region (Figs. 5,6, Plate XX). The cortical initials are less in volume compared to the stelar initials. The peripheral region of the root cap is easily distinguished from the columella by the kappe type of cell arrangement and the degree of stainability.

A true epidermis is absent. As the outer layers peel off the underlying layers serve the function of a protoderm. The colorless cells of the periphery in the mature embryo show accumulation of some colored contents with the development to a seedling. There is no clear demarcation between the cortex and the peripheral part of the root cap. The inner cortical cells give rise to the endodermis composed of colorless cells, far to the proximal side of the root initials.

Root-shoot transition

The entire process of germination upto the dropping of the seedcoat takes nearly forty days after seed wetting. The number of cotyledons ranges from 7-12 and in some cases the

adjacent cotyledons may be joined laterally. The first order needless are not visible in sections of 34 day old seedlings.

The hypocotyle is circular in outline and maintains a uniform thickness to the base of the cotyledons. The hypocotyle in transection shows a uniseriate epidermis beneath is the cortex with 5-7 cell layers. The endodermis and the pericycle are not distinct (Fig. 5, Plate XXI). The stele may be triarch or tetrarch with radial vascular bundles and exarch xylem. Towards the outer side, on the radius of the phloem, there is a colorless mass of cells. These according to Chauveaud (1902) and Wilcox (1962) are the precursory phloem while Schopf (1943) refers to them as the procambium.

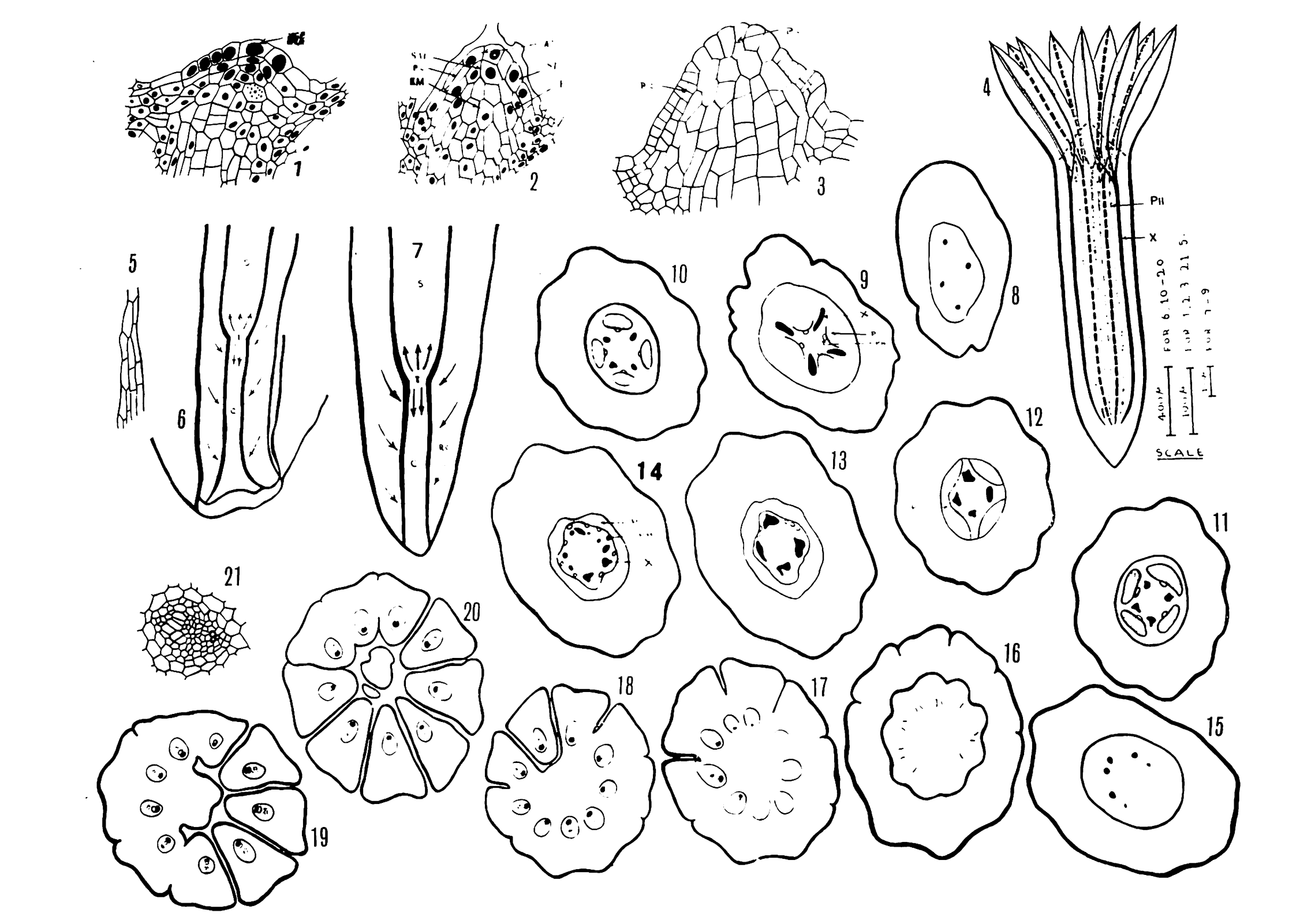
The cambium is two-layered and is seen between the phloem and the precursory phloem, extending upto the pericycle and separating the xylem from the phloem, and the precursory phloem. Pith is seen in the centre. All the layers of the hypocotyle extend into the root and are common except that the precursory phloem is less developed, Xylem is small and the epidermis is not true. The phloem and the precursory phloem differentiate slightly proximal to the root initials.

The vasculature in the root constitutes four xylem and four phloem patches (rarely three also, Fig. 6, Plate XXI). It undergoes the following changes upwards. The xylem is represented by 2-3 cells that shows no distinction into proto and metaxylem. Phloem could not be distinguished at 2050 μ or above the root initials (Fig. 8, Plate V). At 4500 μ the

PLATE V. Explanatory note for Figures 1-21.

1. Median L.S. of shoot apex of dormant mature embryo.
2. Median L.S. of shoot apex of embryo 1 hr after seed wetting.
3. Median L.S. of shoot apex of 34 day old seedling.
4. Three dimensional picture of seedling showing vasculature.
5. Körper divisions in peripheral region of root cap.
6. Median L.S. of radicle of mature embryo.
7. Median L.S. of root of 34 day old seedling.
8. Transection of root 2050 μ above root initials.
9. Transection of root 4,500 μ above root initials.
10. Transection of root 59,900 μ above root initials.
11. T.S. of hypocotyl.
12. T.S. of hypocotyl 825 μ below the shoot apex.
13. T.S. of hypocotyl 555 μ below the shoot apex.
14. T.S. of hypocotyl 465 μ below the shoot apex.
15. T.S. of hypocotyl 375 μ below the shoot apex.
16. T.S. of cotyledonary node.
17. T.S. of hypocotyl 225 μ below the shoot apex.
18. T.S. of hypocotyl 120 μ below the shoot apex.
19. T.S. of hypocotyl 80 μ below the shoot apex.
20. T.S. of hypocotyl 30 μ below the shoot apex.
21. Vascular bundle in the cotyledon.

RB - Rib meristem
PC - Periclinal division
NP - Needle primordium
SAI - Subapical initials
AI - Apical initials
P - Peripheral zone
X - Xylem
PH - Phloem
PP - Precursory phloem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xylem patches widen and show the exarch condition and phloem also becomes evident (Fig. 1, Plate XXI). At the same time the precursory phloem appears between two adjacent xylem patches and towards the outside of the phloem the cambium being between the phloem and precursory phloem (Figs. 10, 11, Plate V).

At a depth of 1100 μ below the shoot tip, the xylem phloem and precursory phloem are well developed (Fig. 11, Plate V; Fig. 2, Plate XXI). The same structure is maintained in the hypocotyle 825 μ below the shoot apex (Fig. 3, Plate XXI). Just below the cotyledonary node (Fig. 12, Plate V), the xylem and phloem widen tangentially with the precursory phloem at its zenith. Below the cotyledonary node each xylem and phloem patch may bi- or trifurcate (Fig. 15, Plate V) and the total number of branches is equal to the number of cotyledons. By this time the four patches of the precursory phloem unite with each other to form a cylinder around the xylem and phloem strands (Fig. 14, Plate V). At the cotyledonary node each xylem and phloem group enters the nearest cotyledon (Figs. 17, 18, Plate V) where the phloem occupies an abaxial position to the xylem and forms the primary vascular bundle (Figs. 19, 20, Plate V). At the cotyledonary node the precursory phloem divides into branches and each branch enters a cotyledon along with the xylem and phloem occupying an abaxial position, to the phloem. In the cotyledon the primary vascular bundle consists of 2-3 xylem trachieds on the adaxial side and the precursory phloem on the abaxial side with the phloem lying

in between the two (Fig. 21, Plate V). There is no direct vascular connection between the hypocotyle and the shoot apex. Hence the shoot and root vascular are independent with the hypocotyles structurally similar to the root.

4.6 TRANSITION OF EPHEDRA FOLIATA SHOOT APEX FROM THE VEGETATIVE TO REPRODUCTIVE PHASE

The xerophytic gymnosperm Ephedra is represented in the desert areas of Rajasthan by E. foliata. Of the total 40 spp. only 4 are reported from India (Mehra, 1950). Pearson (1929) reported in E. foliata, E. americana, and E. intermedia, the presence of male and female flowers in the same or in different inflorescences of the same plant. But E. foliata that grows in Pilani is dioecious as was reported previously by Subramanyam (1953).

Mehra (1934) reported that the flowering time of E. foliata in Punjab is March-April while those that grow in Agra flower in January-February. Mulay (1941) found the plants of E. foliata in Sind and Karachi flowering throughout the year. It was observed that during three consecutive years of the present investigation, the male plants of this region flowered earlier than the female ones. The male plants showed symptoms of flowering from October end to mid November. The female flowers were discernible by the beginning of December.

The inflorescence is typically a panicle with decussate branching system modified by excessive suppression of internodes.

The male spikes are borne on shoots that arise in the long axis or branches of the current year or of the previous season. They arise occasionally from the dormant buds of old stems too. The inflorescence is a single spike or is made of a few branched and sessile spikelets or may be richly branched. The spike or cone consists of several pairs of bracts arranged in 4-6 rows most of which bear slender and sessile flowers in their axils. Each flower consists of a pair of bracteoles and an antherophore. The bracts of successive rows alternate. The antherophore bears 3-4 anthers (at its distal end).

The female inflorescence is more sparsely branched, sessile, and terminal or axillary, bearing 3-4 pairs of bracts that increase in size from below upwards and having a group of 2-3 flowers, or occasionally solitary (Fig. 1, Plate XXII). The female flower consists of a central ovule protected by two investments, the outer husk like (outer integument) and the inner membranous (inner integument).

Ephedra the sole member of the Ephedrales, an order considered of evolutionary importance, deserves a critical appraisal of its ontogenic aspects. A few attempts that centre around the vegetative phase were made earlier. The shoot apical organisation of the vegetative buds of different spp. of Ephedra studied by Deshpande and Bhatnagar (1961), Gifford (1943a), Dayes Dujeu (1957), Fagerlind (1971), Pillai and Pillai (1974) is tunica corpus. Pillai and Pillai (1974) reported that the organisation is the same in E. foliata

without seasonal variations. However, the change over of the apex from vegetative to reproductive was left unattempted except the efforts of Fagerlind (1971).

The present investigation on the vegetative apices of E. foliata further substantiates, some of the observations of Desnpande and Bhatnagar (1961) and Pillai and Pillai (1974).

The apex is conical with an average height of 132 μ and width of 120 μ (Fig. 2, Plate XXII). The apex can be interpreted in terms of the tunica-corpus theory. The tunica is single layered (Fig. 5, Plate XXIII). The corpus is distinguishable into the corpus initials, a flanking zone and pith mother cells zone in both the male and female plants.

In their observations of 120 apices not even a single pericline was noticed in the tunica layer by Pillai and Pillai (1974). In E. altissima, Gifford (1943) observed only one pericline among 40 permanent shoots. Thus the genus Ephedra exhibits a tunica in the strict sense of the term. The cells of the tunica are non-vacuolate and have prominent nuclei (Fig. 5, Plate XXIII).

A zone of 3-7 cells proximal to the tunica, at the summit of the apical dome, constitutes the corpus initials. The cells are densely stained and smaller in size than the cells of the peripheral zone. Divisions in this zone give rise to the flanking and pith mother cells zones.

The cells of the flanking zone are comparatively big and densely cytoplasmic (Fig. 2, Plate XXII). The flanking zone may consist of 2-4 rows of cells and shows irregular divisions during leaf initiation.

The flanking zone is distinguishable by the denser staining and less vacuolation from the central zone of pith mother cells. The leaf primordia originate from the flanking zone, from both tunica and corpus.

The pith mother cells zone gives rise to the pith by divisions perpendicular to the long axis of the shoot. Unlike the conifers, the shoot apical cells of Ephedra are devoid of colored contents. The first indication of vascular differentiation is shown at the second node at the points of leaf origin.

The reproductive apex is visible to the naked eye only with the development of the overarching brown colored bracts, symptomatic of the transitional apex. The leaf is distinguishable from the bracts by the greater rate of growth and size. The bracts, unlike the leaves, show a tendency to overarch the apex. The presence of glandular cells in the epidermal layer is a common feature in both.

4.6.1 Female Plant

With the advent of the reproductive phase, the shoot apex of the female plant exhibits a conical to globose shape. The tunica retains its discreteness while the corpus shows

divisions in several planes. Fig. 3 of Plate XXII shows an apex in the midstage of bract production. Two pairs of bracts have already formed and the surface layers are getting ready for the formation of the third pair. The figure shows the floral meristem exhibiting pericline in corpus cells also. The cell multiplication causes the tunica layer to bulge out to form a buttress. The globose nature of the apex is retained in the course of bract production. Thus the bract production is similar to the leaf production in having the dual origin from the uniseriate tunica as well as the corpus.

Usually four pairs of bracts are produced from the four nodes proximal to the apex. While a vegetative apex shows distinct nodal plates from the third node downwards, the transitional apex lacks them even at the level of the first pair of bracts i.e. at the fourth node. During the production of bracts the pedicel does not elongate.

Concomitant with the inception of bracts, the tunica shows a tendency to exhibit periclines (Fig. 4, Plate XXII).

Fig. 5 in Plate XXII shows the inflorescence apex in the final stages of bract production. At this stage the tunica shows three cells that have undergone periclines. The apex cannot be said to exhibit the tunica corpus organisation at this stage. Subsequent development changes the apex to a homogeneous mass of tissue. Later divisions are mostly in the radial plane that result in a hemispherical dome. Continued

radial divisions of the outer layers give a fan like appearance as is seen in the vegetative apex of Zamia (Johnson, 1951) (Fig. 6, Plate XXII).

The inflorescence at this stage shows a reorganisation in cellular pattern. A mantle core configuration can be applied successfully in preference to the tunica corpus organisation. The outer 4 to 5 highly stained, large nucleate cell-layers form a zone distinct from the inner highly vacuolated and lightly stained cells.

Further development and polarization of activity in the inflorescence apex triggers the initiation of flower production. The flattened apex shows concentration of mitotic activity at two distal poles (Fig. 1, Plate XXIII). Similarly, the central cells of the two poles undergo elongation. When the activity at the two poles intensifies a dichotomisation of the apex ensues. The two centres of activity are the floral meristems, both or only one of them develop into the flower.

The floral meristem shows a considerable number of periclines in the outermost layer (Fig. 4, Plate XXIII). The bracteole or the outer integument arise as a small hump of the floral meristem (Fig. 3, Plate XXIII). It originates not only from the surface layer but from the internal cells also. As the flower axis does not show any internodal growth, the bracteoles stand almost at the base of the axis and the ovule is subtended by them.

The inner integument appears to originate as a ring around the central meristem and is seen to be concrescent with the nucellus. The macrospore cell is seen to originate rather late, only after the elongation of the inner integument as a tube. A rudiment of the central axis is seen in between two flowers (Fig. 4, Plate XXIII).

4.6.2 Male Plant

In the male plant, the inflorescence bud is recognisable by the greater number of bracts that surmount the inflorescence apex. At the inception of the reproductive phase, the bract primordia show increased activity as compared to the foliar primordia. The origin of bract is similar to that in female strobilus.

Two to three bract pairs, formed initially are sterile. Just before the inception of the fertile bracts or even after all the bracts have been produced, the inflorescence apex retains a hemispherical shape (Fig. 6, Plate XXIII). The cells of the surface layer of the inflorescence apex may show elongation in the direction perpendicular to the long axis of the plant. It differs from the vegetative shoot in having a homogeneous mass of cells under the outer surface layer that exhibit periclines. Any strict delimitation of the tunica and corpus zones will be unrealistic as there is little difference in stainability and activity (Fig. 1, Plate XXIV). Hence a mantle core organisation can be applied.

Each floral primordium is subtended by a bract. In the initial stages the development of the bract and floral primordia are similar. Both originate from the surface as well as the subsurface layers. The bract soon after initiation thins down and elongates by predominant periclinal divisions of the cells arranged in two or three longitudinal rows (Fig. 2, Plate XXIV).

The floral primordial initiation is indicated by the denser staining of 6 to 9 cells at the periphery. In some cases 2-3 subdermal cells show great stainability and increase in size (Fig. 1, Plate XXIV). These cells later bulge out to form a lenticular area of meristamatic cells. This develops into a rounded mass and immediately the bracteole formation starts.

The anterior bracteole arises first followed by its counterpart on the posterior side (Fig. 2, Plate XXIV). Thus the bracteoles show an anterio-posterior arrangement. Even when this development is going on in the proximal part of the inflorescence, bract and floral primordia formation continue in acropetal sequence. The inflorescence apex shows a lightly stained inner zone surrounded by a mantle of 3-4 layers of densely stained cells. Once bracteole formation starts, the rest of the meristem shows a gradual decrease in stainability from the outer to the inner layers. The bracteole arises from the outer surface layer alone.

Further development results in a flattening of the antherophore primordial tip (Fig. 3, Plate XXIV). Polarization of activity in a few centres in the subsurface layer indicates the initiation of anther. A tunica corpus differentiation cannot be attributed at this stage. The centres of polarized activity assume a lobed or palmate condition (Fig. 4, Plate XXIV). These lobed structures correspond to the median and lateral lobes of the anther primordia. Concomitant with this elongation of the axis is also noticed. No further development of the sporangia from the anther could be traced. The anther is globose with a less stained outer layer and densely stained inner cells. There is no indication of a rudiment of the apex of the shoot axis. Thus it rules out the possibility of a connation of two microsporophylls as is suggested by Eames (1952). Further it is difficult to provide ontogenetic evidence to agree with Eames (1952) in his view that the four anthers belong to two whorls.

4.6.3 Distribution of Chemical Constituents

There is uniform distribution of total proteins in the vegetative apex (Fig. 5, Plate XXIV). The nucleoli are distinct in materials that are tested for proteins. Bromophenol staining does not reveal any zonation. The nuclei are densely and cytoplasm weakly blue colored.

The uniformity of protein distribution continues during the bract production. The apices of the male plant show

show prevalence of uniform distribution till the elongation of the sporangiophore, but the intensity of staining increases by this time (Fig. 7, Plate XXIV). The anther primordia exhibit denser-staining of all the cells of the globose apex with the exception of the surface layer.

In the female plant, the inflorescence apex shows uniformity in staining pattern of proteins, till the bract production is over. After this three to four outer cell layers show higher cytoplasmic staining (Fig. 6, Plate XXIV). Staining becomes uniform again with the production of inner integument.

The vegetative as well as transitional apices show stainability with periodic acid - Schiff's reagent (Fig. 6, Plate XXII; Fig. 1, Plate XXIII). The insoluble polysaccharides appear light red. The cell wall polysaccharides are more, by the time flowers show signs of maturation. In both vegetative and transitional apices the cytoplasmic staining is uniform. The cells which are about to divide show denser staining. Starch granules are not noticed in the meristem proper of the vegetative apex but occasionally seen in the foliage and internodal regions. In the male flower starch granules accumulate in the stalk cells after the internodal elongation of the sporangiophore. Commensurate with the production of bracts some of the proximal cells of the apex show grains of polysaccharides in both male and female inflorescences. The inflorescence apex of the male plant shows 3 or 4 outer layers of cells more densely stained than the

inner ones.

A differential staining patern is noticed with the use of the metachromatic dye Azure B. The faint green blue colored DNA could be distinguished from the deep blue RNA after staining. The xylem elements react with the stain to give a green coloration. The nucleolus is very well distinguishable and amounts to 2 to 3 per cell (Fig. 1, Plate XXV). A direct correlation can be drawn between the nucleolar size and amount of RNA. Cells with greater nucleolar size show more amount of RNA. In both sexes, the outer two layers of the vegetative apex show intense staining, while a uniform staining is observed in the bract producing apex. With the approach of final stage of bract production there is uniformity of staining in the outer 3 to 4 layers but differs from the inner ones in both sexes (Fig. 2, Plate XXV). The outer layers show more of RNA. The inflorescence apex of the male plant shows denser staining at the apex and also in some localised areas on the sides of the inflorescence, which are the future sites of the bract or floral meristem. Fig. 5 of Plate XXV shows the more densely stained flank cells surrounding a less stained rib meristem region that narrows distally. Here the metabolic activity of the apex is again confined more to the corners, the surface layer itself showing the variation of its centrally disposed cells and the distal ones. The surface cells as well as the cell disposed in the humps stain more intensely for RNA than the rest of the cells.

After perianth production all cells of the lenticular meristem of the male flower and two to three outer cell layers of the female flower show bigger nucleoli and greater RNA content (Figs. 3 and 6, Plate XXV). But the uniform stainability of the male flower is retained till internodal elongation of the sporangiophore. Concomitantly the globose head of the anther maintains an outer stained surface layer which stands out from the inner highly stained cell mass (Fig. 4, Plate XXV). The nucleoli of the outer surface layer are initially as big as those of the inner cell, but later gets reduced. Thus the inner cells show large nucleoli and also highly stained cytoplasm.

With Feulgen staining, wherever nuclei are present, DNA is distinguished by the prominent cherry red color. In all the transitional stages, regardless of the zones nuclei exhibited rather uniform staining (Plate XXVI). Nuclei during division showed greater avidity for the stain.

Based on the orientation of the nuclei, the plane of division of the tunica cells can be easily traced (Fig. 1, Plate XXVI). With the gradual approach of the reproductive phase (when the bract production is over), the nuclei arranged in discrete layers in the distal region of the female inflorescence indicates the mantle-core configuration reported earlier (Fig. 2 and 3, Plate XXVI). Nuclei in all stages of division are seen. The nuclei of the outer and inner cell layers of the male inflorescence and flower apices do not show any variation in staining pattern (Figs.5,6,

Plate XXVI).

As the cells show uniform stainability, no zonation can be distinguished in the vegetative apices that were stained for histone localisation (Fig. 1, Plate XXVII). The nuclei are also stained. The cytoplasm shows weaker staining (Fig.2, Plate XXVII). During the bract production both the male and female inflorescences show greater staining at the loci of young primordia (Fig. 3, Plate XXVII). The apex of the male inflorescence shows 2-3 well stained layers of cells enclosing an inner zone of weakly stained cells. With further development, a uniform staining pattern is exhibited by the young antherophore as is seen in Fig. 5, Plate XXVII. The uniform staining pattern noticed in the female inflorescence apex after bract production (Fig. 4, Plate XXVII) is disturbed. Later, 3-4 outer layers of cells exhibit deeper staining than the inner ones with the initiation of outer integuments (Fig. 6, Plate XXVII).

Enzyme localisation is helpful in studying the floral details as the structural changes are preceded by enzymatic activity. Transitional buds of Ephedra were subjected to the study of enzymes of which peroxidase and acid phosphatase gave good results.

For peroxidase localisation, benzidine was used as hydrogen donor. In the presence of dilute H_2O_2 colorless benzidine is oxidised to benzidine blue by peroxidase which decomposes the quinonediimine to benzidine brown. It is

interesting to note that in all the vegetative and reproductive apices studied the enzyme does not enter the apex, but reaches to the level of the youngest lateral appendages (Figs. 1,2,3 and 4, Plate XXVIII). Staining is quite prominent in the nodal region. The region of provascular tissue also exhibits intense distribution of the enzyme, irrespective of the floral stage. The vegetative apices do not show any variability from the transitional ones as regards the distribution of the enzyme. In the Fig. 4, Plate XXVIII, the pathway of the enzyme is quite distinct in the vascular region all along the length of the bract.

Acid phosphatase was demonstrated in the region of corpus initials of the vegetative apices of the female plant. The tunica layer and flank meristem shows weaker staining (Fig. 5, Plate XXVIII). The transitional stages that lead to inflorescence and flower show a reverse situation with the phosphatase localisation becoming denser in the flank meristem and surface layer cells (Fig. 6, Plate XXVIII). 3-5 outer layers of cells exhibit deeper staining.

CHAPTER V

DISCUSSION

5.1 STRUCTURE OF THE GYMNOSPERM SHOOT APEX

Gymnosperms have attracted the attention of botanists during the past two centuries. Various theories of organisation have been put forth with regard to the apical meristem of plants, some of which are applicable to the interpretation of flowering plants alone. In the present state of knowledge it would not be advisable to depend on a single theory for the interpretation of the shoot apical organisation of all plants.

Nägeli's (1844) apical cell theory still holds true for some Pteridophytes but fails to accommodate other vascular plants. Strasburger (1872) tried to apply Hanstein's Histogen theory (1868) with some modifications to interpret the shoot apices of some gymnosperms. But later workers did not lend support to his view.

Schmidt's (1924) tunica-corpus theory has been successfully applied mainly to angiosperms and to a few advanced members of gymnosperms. Superimposition of this theory by the cytohistological zonation theory (Foster, 1938) has been attempted, as the two are complementary rather than antagonistic. A combination of these concepts has been found to be useful in the interpretation of shoot apices in general,

and those of the gymnosperms in particular.

The French school of anatomists led by Camefort (1950, 1956), Buvat (1952) applied the concept of cytohistological zonation somewhat differently from Foster (1938) and others to interpretation of gymnosperm shoot apex. Camefort recognised only three zones. A zone apicale corresponding to the combined apical initial and central mother cells zones of Foster (1938) was considered to be meristematically inactive. The second zone anneau initial corresponded to the peripheral tissue zone or flank meristem of Foster. The third zone located below the zone apicale and surrounded laterally by anneau initial was called meristem medullaire equivalent to Foster's rib meristem (in angiosperms meristem d'attente). Due to overwhelming criticism, the theory was later revised by Buvat (1955) who conceded a few mitoses in the meristem d'attente zone. He claimed that the relative role of mitoses was minimal in relation to the role played by the anneau initial. The validity of the theory continues to be challenged in recent years by the application of modern techniques such as autoradiography (Gifford, 1960; Clowes, 1959), time-lapse photography (Ball, 1960), etc., and the ideas of the authors of the theory are also undergoing progressive evolution.

Most of the anatomical studies on the apices of gymnosperms and angiosperms conducted during the last two decades lend more support to the theory of cytohistological zonation. Furthermore, the histochemical studies of Fosket

and Miksche (1966) and Riding and Gifford (1973) bring out that the distribution of enzyme systems and metabolites in the various regions of the gymnosperm shoot apex supports the cytohistological zonation theory. In the present attempt the study is extended to some more gymnosperms which further substantiates the zonation theory.

5.2 TYPES OF APICAL ORGANISATION IN GYMNOSPERMS

With the accumulation of voluminous information on the organisation of shoot apex, scientists tried to classify the vascular plants based on their shoot structure. Based on the facts available till that time, Popham (1951) grouped the vascular plants into seven types, of which four types included gymnosperms. Recent studies show that his classification appears to be artificial and unattractive for the fact that apices of advanced gymnosperms like *Ephedra* and *Gnetum* are included under the *Abies-Cryptomeria* type. Moreover, he has made use of the data of the apical structure of the older workers, which are not very accurate, as shown by later studies with more advanced techniques. For example, he followed Koch's (1891) description of the shoot apex of *Pinus strobus* that says that pericliny occurs at the summit only. But recent works including the present one on various pines have proved beyond doubt that the surface layer of the shoot apices of *Pinus* shows periclinal divisions all over.

During his comparison of gymnosperm apices, Johnson (1951) distinguished four types, viz., the Cycadophyta type,

the Ginkgophyta type, the Coniferophyta type and the Tunica-corpus type. Sacher (1954) divided coniferales into three groups based on their apical structure: (a) Ginkgoid type (Pinaceae), (b) Taxoid type (Taxodiaceae, Cupressaceae and Taxaceae), and (c) Araucarioid type (Araucariaceae). Pillai, A. (1962) reaffirmed the occurrence of all these types in Coniferales.

In the light of the present study, Sacher's (1954) contention regarding the classification raises some doubts. Studies on Taxus baccata apices show that there is no stable surface layer. For this reason members of Taxaceae cannot be considered as distinct from members of Pinaceae in the basic architecture of the shoot apex. However, Sacher (1954) has considered members of Taxaceae along with Taxodiaceae and Cupressaceae members that do exhibit a stable surface layer on the flanks, to form the Taxoid type (Plate VI). Further, some members of Pinaceae such as Picea and Cedrus cannot be considered along with the genus Pinus as the former two exhibit a surface layer that shows a few periclines but no oblique divisions. Such a surface layer cannot be identified in Pinus species as divisions in all planes occur all along the length of the outer layer of cells.

Sacher (1954) has made a distinction between the hard pines (2-3 needled) and soft pines (5 needled) based on the structure of the shoot apices. He says that there is an evolutionary trend towards a reduction in the diversification of the tissue zones among the various members of Pinus. He reports that Pinus lambertiana, a soft pine, exhibits a sharp

"Evolutionary advancement seems to have involved a refinement of the meristem, becoming simpler with less diversity in zones".

The shoot apices of the more advanced gymnosperms show a stratified surface layer, a situation met with in angiosperms generally. The stratification of the surface layer becomes clear by the apical initials losing their characteristics and by the elimination of periclinal divisions from the surface cells.

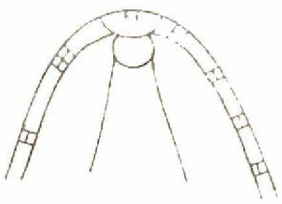
The apical initials are quite distinct in the primitive families like Cycadaceae and Pinaceae. With progressive advancement the apical initials show reduction in size and are difficult to be recognised from the neighbouring cells. Such a situation is noticed in Ephedra. Again, a gradual tendency for the surface layer cells to divide only anticlinally is exhibited by some families, a situation which leads to a tunica-corpus organisation. This status is attained by some families of Conifers (Araucariaceae, Pillai, S.K., 1964) and Ephedrales (Pillai et al., 1972, Pillai and Pillai, 1974).

The apex of Cycas circinalis is unstratified with the occurrence of mitosis in the peripheral layer in all planes. This feature seems to be common to all Cycads and Ginkgo as similar organisations are reported by Foster (Cycas revoluta 1940, Dioon edule 1941b, Microcycas calocoma 1943, Ginkgo 1938) and Johnson (Encephalartos, Bowenia and Macrozamia 1944b) in other cycads.

1 CYCAS CIRCINALIS

2 GINKGO BILOBA

3 PINUS ABIES & TAXUS



4 PICEA & CEDRUS
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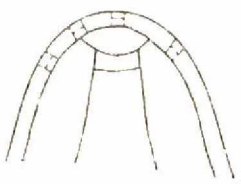
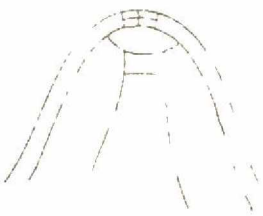
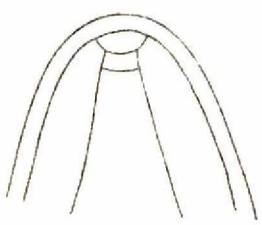


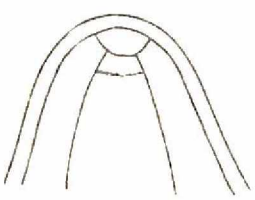
5 PODOCARPUS
PL 41, SR 1953a



6 CUPRESSUS



7 ARAUCARIA
PILLAI, SR 1964



8. EPHEDRA
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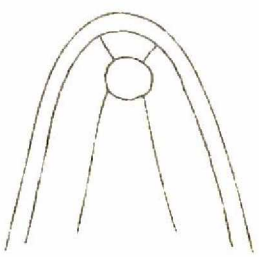
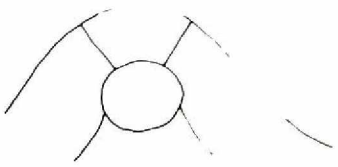


9 GNETUM
PILLAI & PILLAI

PLATE 6 DIAGRAMATIC REPRESENTATION OF THE DIFFERENT TYPES OF ZONATION IN THE SHOOT APICES OF GYMNOSPERMS



1. CYCAS CIRCINALIS
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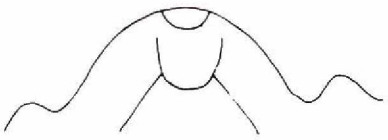
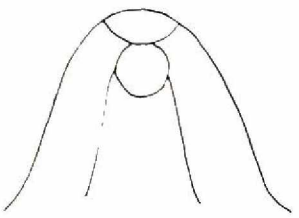
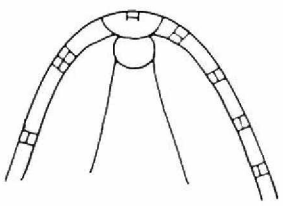


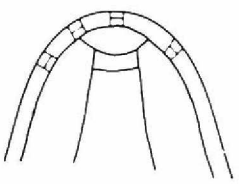
2 GINKGO BILOBA
FOSTER 1938



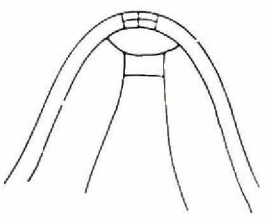
3 PINUS ABIES & TAXUS
(TEXT)



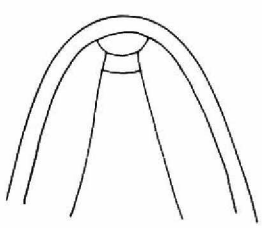
4 PICEA & CEDRUS
(TEXT)



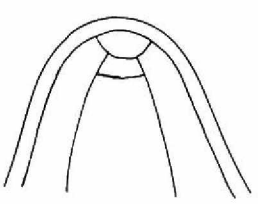
5 PODOCARPUS
PILLAI, S.K. 1963a



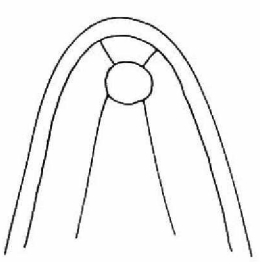
6 CUPRESSUS
(PILLAI, S.K. 1963b)



7. ARAUCARIA
PILLAI, S.K. 1964



8. EPHEDRA
(TEXT)



9 GNETUM
PILLAI & PILLAI [illegible]

PLATE 6 DIAGRAMATIC REPRESENTATION OF THE DIFFERENT TYPES OF ZONATION IN THE SHOOT APICES OF GYMNOSPERMS

The Coniferales, i.e., Pinaceae, Podocarpaceae, Taxodiaceae, Taxaceae and Cupressaceae represent transitional stages between the Cycads and the tunica-corpus organisation met with in Araucariaceae, Gnetales and Ephedrales. Among the various members of Pinaceae, shoot apices of Pinus have been subjected to a thorough study. Sacher (1954) recognised four zones in the apices of P. lambertiana and P. ponderosa viz., the surface layer, the central mother cells, flanking and pith rib meristem zones. The same zonal pattern was reported by later workers for the interpretation of other pines (Tepper, 1966; Fosket and Miksche, 1966; Hanawa, 1969b; Curtis and Popham, 1972; Riding, 1972; and Pillai and Pillai, 1974). The present study on Indian pines further substantiates that the zonation is more or less similar in all pines and that their unstratified apices exhibit periclinal, anticlinal and oblique divisions in the cells of the surface layer. Structures resembling this are reported in Taxus baccata, Abies pindrow, Larix (Schopf, 1943), Sequoia (Sterling, 1945) and Abies concolor (Parke, 1959). But in members like Picea smithiana and Cedrus deodara, a surface layer can be distinguished on the flanks, as oblique divisions are uncommon and predominant anticlines with a few periclines decide the cell lineage.

In the other families of Conifers like Podocarpaceae, Cupressaceae and Taxodiaceae, there appears to be a tendency for gradual elimination of periclines from the flanks upwards at certain periods of the full annual cycle.

The apices of Podocarpaceae (Pillai, S.K., 1963; Pillai and Pillai, 1974) exhibit periclines in the apical initials and the surface layer on the upper flanks during active growth, while during the dormant stages the surface layer simulates a tunica. In Callitris robusta (Cupressaceae) and Cryptomeria japonica (Taxodiaceae) Pillai and Pillai (1974) reported periclines only in the apical initials and upper flanks of the surface layer. In Cephalotaxaceae and some members of Cupressaceae and Taxodiaceae this tendency is more pronounced where even in the active shoot, periclines occur only in the few apical initials at the summit and not in the surface layer on the flanks (Cupressus sempervirens, C. torulosa, C. macrocarpa (Pillai, S.K., 1963), Thuja orientalis, T. compacta, Juniperus chinensis (Pillai, A., 1963), Taxodium distichum (Cross, 1939), and Cephalotaxus drupacea (Singh, 1961) (Plate VI).

The tendency towards the elimination of periclines reaches the climax in Araucariaceae. In Agathis lanecolata Sterling (1958) reported the occurrence of a tunica with a few periclines. But a reinvestigation of the genus by Pillai and Pillai (1974) showed the complete absence of periclines in the surface layer. In Agathis palmerstonei and A. alba, Pillai and Pillai (1974) report the occurrence of double and single layered tunica respectively. In his examination of 120 apices of Araucaria columnaris, Pillai (1964) observed not even a single pericline in the surface layer. Griffith (1952) reported a similar situation in four species of Araucaria.

The situation in Gnetaceae and Ephedraceae is not different from that of Araucariaceae. In Gnetum gnemon, Johnson (1950) reported the presence of a tunica with a few periclines. But in G. ula, Pillai and Pillai (1974) failed to notice any pericline. Different species of Ephedra studied by Gifford (1943), Deshpande and Bhatnagar (1961), Fagerlind (1971), Pillai and Pillai (1974) and the present attempt reveal that the occasional periclinal division at the summit cells is more the exception than the rule.

Thus, starting from the primitive Cycads that exhibit a naked corpus-like structure, passing through the different families of conifers with partially stratified apices, we reach Araucariaceae, Gnetaceae and Ephedraceae, where the surface layer of the shoot stabilizes to simulate a tunica. Furthermore, the tendency for the elimination of periclines from cells of the surface layer starts from the lower flanks to the upper flanks and finally to the summit cells.

Another trend of specialisation that can be observed within the gymnosperms is the gradual reduction in the size of the central mother cells and the establishment of the subapical initials. A fairly large group of central mother cells is present in the primitive cycads and Ginkgo. At the higher levels of heirarchy such as conifers and Ephedrales, specialised subapical initials, which are much fewer in number are seen. The only case of a throwback is the presence of central mother cells along with subapical initials in Gnetum ula (Pillai and Pillai, 1974).

The progenitor of the pith in gymnosperms may be a zone of pith mother cells or a pith rib meristem. An extensive area of pith rib meristem with predominantly transverse divisions seen in Cycas circinalis, Pinus spp., Cedrus, etc. give rise to the pith. It is considerably reduced in depth and size in many of the conifers and is called the pith mother cells zone. Here again, in actively growing shoot apices, a pith rib meristem can be distinguished in some species. In Ephedra a distinct zone of pith mother cells arises from the base of the corpus initials. This is yet another line of advancement where the extensive pith rib meristem gives way to the considerably reduced zone of pith mother cells.

5.4 SEASONAL VARIATIONS IN THE CONIFER SHOOT APEX

As early as 1893, Busse had noticed variations in the size of the gymnosperm shoot apex during the different seasons. Later studies on different members of gymnosperms showed that the organisation too exhibited variations during the annual cycle. Based on the activity of the apex during the different seasons, three growth phases were recognised by Korody (1937) in Abies concolor, viz., (1) rest, (2) growth phase I, characterized by bud expansion and cataphyll production, and (3) growth phase II characterized by needle production. The same growth phases were recognised by Parke (1959) in Abies concolor and Owston (1969) in Pinus strobus. Kemp (1943) followed the seasonality of Torreya buds, and considered the production of cataphyll and needle as

manifestations of the same growth phase that constitutes the new bud formation. Frampton (1960), Singh (1961) and Pillai (1963a) considered the cataphyll production and needle production as separate and recognised four phases in Larix, Cephalotaxus and Podocarpus respectively.

The Conifers may or may not exhibit definite seasonal variations. No definite seasonal variation throughout the annual cycle is reported in some Cupressus spp. (Al Sherifi, 1952), Cupressus sempervirens (Pillai, S.K., 1963a), and Araucaria columnaris (Pillai, S.K., 1964).

Both the Conifers, whose seasonal variations have been studied here, exhibit definite seasonality. Eventhough both the plants were growing under the same geographic conditions, the duration of dormancy happened to be different for these two species. In Picea, dormancy is of a short duration, beginning in December and lasting upto the end of February. Cedrus has a longer dormant period starting at the end of September and lasting upto February. The apices of both plants show a definite rhythmical sequence of volume relations during the annual growth cycle. In Picea and Cedrus the minimum volume is observed during the rest and elongation phase respectively, followed by an increase in the succeeding phase. The maximum size of the apex is attained at the time of organogenesis in Cedrus.

Picea exhibits a tendency towards stabilization of the surface layer as few periclines are noticed during the

rest period. During the rest of the year, periclines occur all over the surface layer. But Shah and Thulasy (1968) found no pericline in the overwintering buds. Their study did not include the annual growth cycle. However, Cedrus exhibits periclines throughout the year in both dwarf and long shoots.

Excluding the efforts of Owens and Molder (1973), none of the earlier workers have followed the pattern of histochemical changes in the gymnosperm apex throughout the annual cycle. The study of Owens and Molder (1973) laid emphasis on the fluctuations of mitotic frequency of DNA content during the different phases of organogenesis in Doughlas fir. No mitosis was noticed in the dormant stage. Cytophotometric studies showed that in mitotic frequency the peripheral zone was much more active than the combined apical initials and central mother cells zone during period of activity.

Frampton (1960) reported the disappearance of starch from the winter buds of Larix decidua. Bachelard and Wightman (1973) found a steep decline in the starch content two weeks earlier to bud break followed by a sudden rise after bud burst in Populus balsamifera. But the present investigations provide somewhat contradictory results. Both Picea and Cedrus exhibit accumulation of insoluble sugars at the shoot apex during dormancy with their appearance in the pith slightly earlier to the onset of dormancy. The granules of polysaccharides disappear from the portion above the crown

with the arrival of spring in April. Hence, it is presumed that they are used up for the elongation of the bud. Furthermore, the polysaccharides in the pith and cortex below the crown play no role in elongation, as the amount of the sugars below the crown remains the same even after elongation.

Histochemical studies made on the two plants during the annual cycle reveal that the meristematic activity is restricted primarily to the flank regions of the bud. Distribution of DNA can be the best indication of areas of high activity. In the median longitudinal section the maximum amount of DNA is noticed in the peripheral region and less in the apical initials, subapical initials and the central tissue. Further, the distribution of total proteins and histones is similar to that of DNA, maximum being noticed in the peripheral zone. The high distribution of protein is an indication of high enzyme activity. The subapical region shows the same degree of stainability for DNA as the apical initials, and hence cannot be considered quiescent.

5.5 CROWN FORMATION AND WINTER DORMANCY

Crown is mostly seen in those gymnosperms that have a definite period of bud dormancy. Plants like Araucaria (Pillai, S.K., 1964), Cupressus (Pillai, S.K., 1963b) and Thuja (Pillai, A., 1963) are reported to lack definite seasonal growth phases and crowns. The only exception is Pinus which possesses definite seasonal variations but shows the absence

of a crown. It is understood from the available literature that the crown is genus specific, as different species of the same genus studied, are reported either to possess it or not.

The present study shows that the entry of vascular elements into the new bud is prevented by the crown. By March-April (i.e.) at bud break, vascular differentiation takes place above the crown and the bud shows signs of reactivity. In his enzymatic studies of the shoot apical meristem of Picea glauca, Vanden Born (1963) noticed high activity of phosphatase around the crown.

In this context, the view expressed by the School of Forestry, Oregon University, led by Lavender (1973) deserves special mention. Their experiments on Pseudotsuga menziesii (Mirb) (crown is reported in Pseudotsuga taxifolia by Sterling, 1946) using gibberellins and different regimes of day length and temperature conditions showed that growth can be initiated in this plant by gibberellins exported from the roots. It was shown that plants kept at 5°C with gibberellic acid treatment can be induced to spring burst as fast as plants kept at 20°C on which the application of gibberellic acid was ineffective. Their earlier experiments showed that gibberellins are produced in the roots. Further, Wareing et al (1971) were able to release bud dormancy of Acer pseudoplatanus by gibberellin application in early winter. Thus, it is plausible that the upward translocation of gibberellin-like substances from the lower region to the new

bud may be prevented by the crown to induce dormancy.

Further, the colored contents richly present in the pith cells above the crown in Picea during dormancy also deserves attention. The most convincing evidence that dormancy regulation may involve an interaction between growth promoters and inhibitors is provided by studies on buds (Wareing and Saunders, 1971). As there are no histochemical methods for localising the hormones and inhibitors, further progress will depend upon the application of analytical techniques.

The crown deserves consideration not only for its supposed role in dormancy, but also for its application in Dendrology. As the crown records one year's growth of the plant and only one crown is formed in an year the age of the plant can be determined by counting the number of crowns from tip to bottom. The length of one year's growth may be defined as the distance along the centre of the pith between two successive crowns. By studying the crown, the up and down portions of a piece of wood can be decided (Venn, 1965).

The present observations on Picea and Cedrus that the crown cells are rich in lignin and pectin contradict the view of Srivastava (1966), who arrived at the conclusion that lignified cells are absent in the pith of gymnosperms. The crown that originates from the pith must have been overlooked by him as he does not mention about the crown in his text. In this context the observations of Romberger and Tabor (1975) that polysaccharides and lignin-like substances accumulate

beneath the apical meristem of Picea abies in in vitro culture, can be meaningful to presume that they are distantly associated with the crown plate.

Some of the reports about the presence of crown seem to be confusing. Sterling (1945a) mentions about its presence in the winter buds of Sequoia sempervirens and refers it in Figs. 9 and 10 of his article which do not show any distinct region that can be designated a crown. Moreover, this thickened area is reported to extend to a length of about one mm in those apices. A similar situation is described in Torreya by Kemp (1943). She thinks that two crowns are formed in a season, a phenomenon not reported by others.

5.6 INTERCALARY GROWTH IN EPHEDRA

Ephedra is unique among gymnosperms in possessing all the three types of meristems, namely apical, lateral and intercalary. The intercalary meristem differs from the other two types as it eventually transforms into mature tissue.

Two types of intercalary meristem are recognized by Jacobs (1947). In grasses it is referred to as "detached meristem", as the meristem is detached from the apical meristem by the gradual maturation of the tissues in between the apical meristem and the region of intercalary meristem. The intercalary meristem is active continuously like its progenitor, the apical meristem, without a period of rest. Jacobs (1947) records the second type in Arachis hypogea which develops a gyno-phore from the short ovarian stalk. Pollination induces

an extension growth of the gynophore by an intercalary meristem. As the intercalary meristem is inactive till the time of pollination and the latter is induced to resume growth, it is called a "resumptive meristem".

The occurrence of an intercalary meristem in the genus Ephedra was earlier noticed by Dayes-Dujeu (1957) in E. monostachya. According to him the differential activity of the lower and upper portions of the internode starts from the first node itself. In fact, he attributes the articulation of the vegetative body into nodes and internodes to the activity of the intercalary meristem. In E. foliata the activity of the intercalary meristem is identified from that of the apical meristem rather late, only by the third or fourth node.

The intercalary meristem in E. foliata occurs somewhat above the base of the internode. The region of intercalary meristem remains unchanged even in older internodes (irrespective of age). This situation is reported in other plants too like Eleocharis (Evans, 1965), Wheat (Ordina, 1952) and Cyperus alternifolius (Fischer, 1970). As the distance it keeps from the lower nodal plexus is the same for different internodes, basipetal activity is unquestionably negligible. This is in contrast to that of Avena sativa where basipetal activity has been reported (Kaufman et al, 1965).

In Equisetum arvense, Golub and Wetmore (1948) reported the intercalary meristem to be ring-shaped and pith formed

from the intercalary meristem. Further, they reported that the intercalary meristem does not cut across the vascular strands. In E. foliata the activity of theintercalary meristem prevails over the pith, cortex and epidermis. In older nodes the intercalary meristem is seen not cutting across the vascular bundles but to laterally join with the xylem parenchyma. The vascular elements at the region of elongation are spiral and annular that can cope up with the process of longitudinal extension. This situation prevails in the region of plants where extension growth takes place (Cutter, 1971).

The vague boundary of the intercalary meristem in the young internodes becomes more and more distinct with the gradual depression of activity. By the 10th internode the intercalary meristem is a thin belt across the internode, and composed of 4-5 cell layers. This is in contrast to other reports of intercalary meristems where their cells mature soon after their activity. In none of the earlier reports regarding the intercalary meristem we meet with a situation where it remains quiescent for years. As the cells possess distinct nuclei and cytoplasm compared to the pitted and lignified cells above and below it, it is plausible that it may have some role in lateral conduction. With these characteristics it is difficult to categorise the intercalary meristem of E. foliata under the groups of "detached meristem" or the "resumptive meristem" of Jacobs (1947).

Internodal growth in E. foliata is not only due to the presence of intercalary meristem but also due to cell elongation. The normal rib meristem cell that measures about 20 μ in length in the apical region undergoes longitudinal extension upto 300 μ. Thus, a 15-fold increment in length is achieved, when compared with 7-10 fold increase in Helianthus (Wetmore and Garrison, 1961) and 16-fold increase in Avena sativa (Kaufman et al, 1965).

Cell division and cell elongation are continuous processes in the meristem of roots and shoots and in internodes that exhibit intercalary meristem activity. This is documented in the studies of Jacobs (1947), Jensen and Kavajian (1958), Erickson and Sax (1956), Kaufman et al (1965), and French and Paolillo (1975). In E. foliata the maximum elongation of the cell is noticed in the central portion of the pith in the internode while cell multiplication is noticed at the basal region of the internode.

Wetmore and Garrison (1961) conducted experiments on internodal growth of Helianthus annuus and noticed that a wave of multiplication starts from the bottom of the internode in an acropetal direction. If the cell multiplication occurs in a wave in E. foliata, one should expect most of the divisional figures at the same phases at a particular region in the internode. Instead, a random distribution of the various phases is noticed all along the length.

Thus, intercalary growth in E. foliata involves growth by cell multiplication due to an intercalary meristem coupled with cell elongation.

5.7 APICAL ONTOGENY OF PICEA SMITHIANA DURING GERMINATION

The seeds are comparable to buds in exhibiting a marked periodicity with the concomitant expression such as dormancy and apical zonation. The ontogenic study of the germinating seed is of interest as we are able to trace the development of the apical meristems from the fully meristematic cells of the embryo.

Shoot Apex

Reports on the occurrence of zonation in the shoot apices of developing embryos and seedlings vary. Spurr (1949) and Gregory and Romberger (1972) reported the presence of cytohistological zonation in the shoot apices of immature embryos of Pinus strobus and Picea abies respectively. Prior to the initiation of cotyledon primordia, the apex of the immature embryo of Picea abies shows a distinct group of subapical cells. A similar zone of subapical cells that are highly vacuolate and weakly stained is noticed in the dormant embryo of Picea smithiana. In Picea smithiana zonation becomes more distinct one month after germination, as in Picea abies (Gregory and Romberger, 1972). The zonate pattern was noticed rather late in the embryonic development of other conifers. Allen (1946) observed in the embryos of Pseudotsuga, a lack of zonatiom in the shoot apex till one

month after germination. In Pinus lambertiana shoot apex exhibited zonation 8 days after germination (Fosket and Miksche, 1966). By 5th day the authors observed a mantle of 2-5 cells with dense cytoplasm and larger nuclei around an internal core of cells with smaller nuclei. The usual zonation pattern with 4 zones was observed very late. Riding (1972) observed zonation in the dormant embryos of Pinus radiata but attributed it to the staining differences of storage products but not to the cell size or state of activity. The zonation with the latter qualifications was noticed in P. smithiana 35 days after germination. Tepper (1964) expressed the view that an initially zonate apex becomes non-zonate later in seedlings of Pinus ponderosa and regains the apical zonation during further development.

From these reports, it can be seen that during seedling development (1) there are marked differences in the time of appearance of the zonation of the apex, and (2) the time taken for stabilization of zonation may vary from species to species.

Root Apex

The radicular apex and the root apex of the seedling exhibit a basically similar configuration. There is a common initiating region from which the columella cells arise distally and the stelar cells proximally. Surrounding this is a hollow cup-shaped group of initials. The cell files produced from this zone broaden distally by 'Kappe' divisions

and abut around the columella head. The proximal cells of this group differentiate into the cortex while the outer files, which proceed towards the tip of the root form the peripheral part of the root cap. In the embryo, however, the 'Kappe' divisions of the protoderm surrounding the hypocotyle give rise to the peripheral part of the root cap. As growth proceeds this zone is replaced by cells of the outer cortical files, the older files from the protoderm being sloughed off evidently.

The concept of Schopf (1943) that the pericolumnar cells near the stelar initials change polarity abruptly, become oriented slantingly and give rise to the cortical files proximally, cannot be supported by the analysis of the cell lineages. This has already been brought out by Pillai,A. (1964, 1966) in the root apices of a large number of conifers and Ephedra foliata. This cell lineage must be a continuation of the activity which started during histogenesis in the embryo.

Root-Shoot Transition

Root-stem transition has been conceived of as a process of rearrangement of vascular strands by splitting, rotation, and fusion (Van Tieghem, 1891; Eames and MacDaniels, 1947). The xylem is described as bifurcating, splitting and rotating through 180° to become endarch while the phloem also splits simultaneously. This situation exists when the vasculature is continuous from the root to the shoot.

However, Picea smithiana does not exhibit such a connection between the shoot and root vasculature. The vasculature is found to be continuous only from the root to the cotyledons through the hypocotyle.

Bonnier (1900) believed that a shift in the xylem pole differentiation and the primary phloem occurs gradually resulting in the transition. This has been reported in some angiosperms such as Tridax procumbens (Padmanabhan, 1968), Amaranthus leucocarpus (Sebastian, 1971), and Hevea brasiliensis (Panikkar, 1974). In the present study the xylem patches enlarge tangentially but the pole of xylem differentiation does not show any shift.

Sterckz (1900) considered that the stem, leaf and root are fundamental morphological entities and that the traces of the cotyledons, leaves and roots are put into contact by mere juxtaposition in the hypocotyl region without any longitudinal connection between the strands. The results reported here, seem to justify the suggestion that the vasculature of the root and shoot are distinct morphologically. Thus, the present study lends support to Thoday's (1939) interpretation of the double origin of the vascular system and not to the unitary system (Eames and MacDaniels, 1947).

5.8 SHOOT APEX OF EPHEDRA FOLIATA DURING TRANSITION FROM VEGETATIVE TO REPRODUCTIVE PHASE

Studies on the organisation of the shoot apex of flowering plants is of considerable practical importance. The study of ontogeny of the different floral parts helps to attribute the origin of the sporogenous cells to particular zones of initiation. These zones may belong to the outer surface layer, second layer or internal cell layers of the meristem depending on the species. During the induction of mutation by chemical treatments, the concentration applied can be appropriately adjusted to get predictable results, if the investigator has a background knowledge about the origin of sporogenous cells. Its importance, in this respect demands greater attention in the case of plants that have a longer period of seed setting, as in gymnosperms.

Studies of cone initiation in gymnosperms lag behind comparable studies of floral initiation in angiosperms. There are reports regarding a comparison of the nature of vegetative and reproductive apices of coniferous plants. But a sequential study of the changes involved in the meristem during the transition to flowering are very few, like those of Hejnowicz (1957), Owens and Pharis (1967), Taillandier (1967) and Fagerlind (1971).

The present attempt to study the sequential changes in the meristem proper of Ephedra foliata may help bridge the informational gap between conifers and angiosperms because of Ephedra's position in the evolutionary ladder.

The transition of a vegetative apex to a pollen cone apex in Cupressus arizonica is marked by the formation of a distinct transitional or branching apex (Owens and Pharis, 1967). This is distinguished by lateral appendages being initiated unusually high on the apex above the last-formed leaf primordia. In Ephedra no such transitional apex is noticed, nor in Thuja plicata (Owens and Pharis, 1967).

In E. foliata the transformation of a leafy shoot into a reproductive one is marked by an enlargement of the shoot apex. This feature has been reported in angiosperms (Philipson,1947, Popham and Chan, 1950, Wetmore, 1959, Gifford and Tepper, 1961, and Molder and Owens, 1973). In angiosperms, during the early stage of transition, just after the increase in size of the apex, internodal elongation occurs (Owens and Molder, 1973; Philipson,1947; Sachs, et al 1959). Hence, elevation of the apex can be considered, a characteristic of the transitional stage. But in Ephedra there is no internodal elongation for the strobilus, but is predominant in the antherophore of the male flower. However, it is rather delayed till microsporogenesis.

In angiosperms, Wong and Comb (1967) noted that cell elongation in the internode is accompanied by the utilization of polysaccharides accumulated in the cells, a view shared by Molder and Owens (1973). This is again prevalent in the vegetative apices of Picea and Cedrus. But a reverse situation is noticed in the reproductive phase of E. foliata. The polysaccharide granules are noticed in the reproductive

apex only after the internodal elongation. Gifford and Tepper (1962) observed a similar situation in the transitional apex of Chenopodium after six days of photoinduction. The polysaccharide granules can be the sites of proplastids which are to differentiate later into chloroplasts and amyloplasts. This conclusion will harmonise with Corporali's (1958) contention that starch grain is always included in the fundamental substance of a very young plastid.

Ontogenic studies show that the tunica may or may not gain or lose layers during the transition from vegetative to inflorescence to floral apex. Vaughan (1955) observed that the vegetative and inflorescence apices in Capsella bursapastoris are structurally similar in his attempt to verify the report of Chakravarti (1953) that an increase in apical layering occurs during the growth of the inflorescence apex. Tucker (1959) noticed two tunica layers in the vegetative shoot apex of Drimys, two in the inflorescence and either one or two in the floral apex. Portulacca grandiflora (Soetiarto and Ball, 1969) exhibits two tunica layers in vegetative apex which fluctuate to three during the early stages of transition and two in the final stages. Bernier (1964) reports a mantle-like zone of superficial layers of cells that accompany the transition to 'prefloral stage'.

Male Plant

The transforming apex of E. foliata, does not exhibit a tunica-corpus differentiation throughout the development.

The tunica-corpus organisation is retained in the male strobilus till the final stage of bract production only. Thereafter, constant disturbance of the surface layer by pericliny is noticed. A mantle-core terminology is more appropriate to the later stages. During the antherophore and anther development less number of pericliny is noticed.

Strobilar development in Ephedra was earlier studied by Strasburger (1872) and recently by Fagerlind (1971). Fagerlind attempted to draw some generalization from the study of six different species, viz., E. alata, E. alte, E. americana, E. altissima, E. compylopoda and E. distachya. In these species Fagerlind (1971) observes the complete loss and reappearance of the tunica layers. Just as in E. foliata, during the formation of perianth and antherophore, he notices the absence of tunica-corpus organisation. He is not able to fix the stage at which the tunica-corpus differentiation ceases. During the final stages of anther development he observes the reappearance of tunica-corpus organisation. In their attempt to study the embryology of E. gerardiana, Singh and Maheshwari (1962) observed a similar situation of reappearance of tunica-corpus differentiation from the time of inception of antherophore primordia.

The development of the dorsal perianth lobe of the male flower prior to the ventral counterpart seems to be common for all species of Ephedra. Fagerlind also noticed the dominant development of the dorsal perianth. Somehow, the present observations are not enough to draw a conclusion

regarding his view that perianth initials at the dorsal side gradually progress around the sides, leading to a ring-like enclosure. In E. foliata it seems that the perianth lobes arise freely, one independent of the other.

Just after the delimitation of the perianth primordia, the apical meristem should represent two primordia if two microsporophylls have coalesced to constitute a single antherophore. This is the view expressed by Eames (1952) regarding the morphology of column. In E. foliata only one primordium is noticed after the inception of perianth, which may indicate the origin of column from one microsporophyll.

Female Plant

The female flower consists of a nucellus enclosed by two investments. The inner one is acknowledged as the inner integument and the outer one as the outer integument or the perianth (Fagerlind, 1971).

After bract production, the inflorescence apex exhibits a transitional reorganisation. A mantle-core terminology is applied to this in preference to the tunica-corpus terminology. A fan-like appearance is exhibited by the floral apex due to the continued periclinal divisions resulting in radiating rows.

Due to the non-availability of young female flowers, Fagerlind's (1971) study was limited to flowers that had already developed the perianth. He shares with earlier workers the view that the perianth soon after its initiation

becomes a closed ring. It originates with greater increase in the dorsal part in E. foliata. But Mehra (1950) noticed in E. intermedia the perianth had parted completely into a right half and left half thus indicating dual origin from two poles.

It seems that the endosperm mother cells (EMC) take their origin at different stages in different species. Fagerlind (1971) reports the formation of embryo sac mother cell prior to the inception of the integument. But in Ephedra foliata this occurs rather late. The embryo sac mother cells are observed only after the development of the integument around the nucellus.

Strasburger (1872), Eames (1952) and Fagerlind (1971) have called attention to the presence of a rudimentary outgrowth of cells between the flowers. This is considered as the inactive apex of the strobilar axis. Its presence is observable in female flowers of E. foliata. Hence it is justifiable to consider the female flowers also to have the same axillary arrangement as the male.

Male and Female Plants in General

The overall results show that the zonation pattern changes in the apex during transition in E. foliata. A true tunica-corpus organisation is met with in E. foliata only upto the final stage of bract production in the flowers of both sexes. This is in contrast to other species of Ephedra studied by Fagerlind (1971) who noticed the reappearance of a tunica-corpus differentiation during anther development.

Generally, stratification seems to be a prelude to the development of inflorescence. In Chenopodium, the apex gradually becomes stratified with 4-5 layers at floral induction (Gifford and Tepper, 1962). Tucker (1959) noticed the same feature in Drimys. In the present study Ephedra also is seen to exhibit the same.

The nucleolar number varies from 2 to 4 per nucleus in Ephedra against 4 to 6 in Jack pine reported by Durzan et al (1971). The nucleoli of all zones in the transitional apex are larger than those of the vegetative apex. This increase in nucleolar volume was noticed by Lance (1957), Gifford and Tepper (1961) and Nougarede et al (1965). Such an increase is also noticed in the axial and central zone more than the peripheral and lateral tunica in Cosmos (Molder and Owens, 1972). The maximum nucleolar size is noticed in Ephedra in the sporogenous cells of the anther. The outer surface cells covering the sporogenous cells also exhibit bigger nucleoli initially but later development shows decrease in size.

The staining pattern of nucleoli by alkaline fast green in all apices is contrary to the findings of Knox and Evans (1966). They reported in Lolium longulatum and Pinus radiata the nucleoli of the shoot apices to be free of histones though they used the same stain, fast green FCF. But Gifford and Dengler (1966) using the same stain had noticed the presence of histones in the nucleolus. Recently, Raju and Ho (1973) reported the same in leafy spurge.

In all stages of Ephedra, the cytoplasm was stained by fast green and the nuclei showed denser staining than the cytoplasm. Positive results of cytoplasmic histones are reported in many plants (Thorpe and Murashige, 1970; Riding and Gifford, 1973). In the shoot apices of leafy spurge, Raju and Ho (1973) report that the cytoplasm has denser stainability than the nucleus in the central distal region during the rapid growth phase.

During the early stage of transition to the female inflorescence the amount of RNA increases followed by the occurrence of stratification. Then the distribution of RNA becomes uniform and the flower initiation takes place. The increase in RNA, noticed in Sinapis, Pharbitis and Lolium (Bernier, 1971) occurring at the time of floral stimulation is designated as floral evocation by Evans (1969). This state of evocation appears to be followed by an increase in cell division.

The staining pattern of the young male inflorescence of Ephedra recalls the pattern of RNA distribution in the spikelet of Lolium temulentum (Knox and Evans, 1969). They report increase in RNA concentration in certain localised areas at the periphery of the longitudinally sectioned inflorescence.

Owens and Pharis (1967) noted in Cupressus species increase in mitotic index in the transforming apex after floral stimulation to be treble that of the vegetative

apex. A visual estimate of the number of divisional figures in the vegetative and reproductive apices of Ephedra foliata gives a comparable increase in mitotic index.

No single role is attributed to the enzyme peroxidase. A varying number of isoenzymes of peroxidase are reported in the different stages of seed germination in Pinus banksiana (Ramaiah et al, 1971). Vanden Born (1963) has reported higher activity of peroxidase at the bottom of the young needle where a zone of intercalary meristem is active. Its presence in the peripheral and rib meristem regions of the shoot apex has been reported by Van Fleet (1959), Vanden Born (1963) and Riding and Gifford (1973). The present observations on Ephedra foliata show similarity with those of Vanden Born (1963) regarding the abundance of peroxidase in the provascular strands. He noticed greater concentration of the enzyme in the provascular strands of young male cones of Picea glauca. A comparable situation is reported in Pinus banksiana too (Ramaiah et al, 1971).

In corn, Van Fleet (1959) could predict in advance the regions of buds in the stem apex by noticing the path of peroxidase. He concluded that peroxidase activity is associated with regions of rapidly dividing cells. Vanden Born (1963) reported that the enzyme can be detected in advance of the subsequent cell division in wound meristem, primordial centres and protophloem and that the enzyme concentration declines after the divisional phase. But peroxidase activity cannot be considered as an indicator of

cell division as it is not seen in the peripheral regions of the shoot apex of E. foliata where maximum divisional phases are noticed in the Feulgen stained apices of similar stages. Further, peroxidase activity is noticed in the mature phloem and developing xylem where no division occurs (Van Fleet, 1959).

Galston and Davies (1969) hold the view that peroxidase activity reflects the ability of a plant tissue to degrade endogenous Indole Acetic Acid since the activity apparently resides in the same protein as Indole Acetic Acid oxidase. But Elkinawy and Raaj (1973) showed that peroxidase activity is not proportional to the Indole Acetic Acid Oxidase activity, nor can it be used as a measure of the tissue's ability to degrade Indole Acetic Acid.

Helper et al (1970) postulate that peroxidase participates in the process of lignification as they are localised, specifically in the secondary wall. They visualize that peroxidase and other enzymes present at specific loci in the cell matrix, would initiate and control the condensation of lignin monomers onto the surface of the cellulose microfibril. This view is further reflected in the works of Hall et al (1974). But the electron microscopic studies of Goff (1975) in the onion roots revealed that there is no lignin deposition in the region showing intense cell wall peroxidase. It is also reported that peroxidase has a role in hydrogen transfer during respiration.

Keeping in view the hypothesis (Van Fleet, 1959) that the continuous peroxidase system observed in phloem forms a connecting link between adult tissues and meristematic regions, the present observation of peroxidase localisation in the vascular regions and nodal plate seems fitting. The peroxidase activity may be involved in providing some continuity between mature and meristematic regions and may function in the transport of specific substances.

The function of acid phosphatase in shoot apices is unknown (Van Fleet, 1962; Fosket and Miksche, 1966; Riding and Gifford, 1973). The present studies on Ephedra foliata show ~~that~~ relatively intense staining for acid phosphatase ~~is noticed~~ in the corpus initials of the vegetative apex. Transitional apices leading to inflorescence and flower show a reverse situation with the phosphatase activity becoming intense in the flank meristem and surface layer cells. The stratified layers of the transitional apex forming the mantle just before the development of the female flowers show denser staining than the cells of the inner core. This pattern of enzyme distribution shows similarity with the shoot apices of Pinus lambertiana (Fosket and Miksche, 1966) during seed germination. The intense phosphatase activity of the central mother cell zone and the weak stainability of the peripheral zone noticed in the shoot apices of Pinus lambertiana till five days after germination are reversed eight days after germination. It is to be noted that in P. lambertiana (Fosket and Miksche, 1966) the stabilization

of the cytohistological zonation occurs only by eighth day after germination. Thus, it is quite likely that acid phosphatase activity is associated with the process of reorganisation and differentiation of cells in shoot apex.

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SUMMARY

The present investigations on the shoot apical organisation of twelve species of different families of gymnosperms show that Foster's (1938) theory of cytohistological zonation can well be applied to interpret it. The vegetative apices of Cycas circinalis are shown to possess four zones, viz., the apical initials, central mother cells, flank meristem and rib meristem. The zonation in all the conifers studied is the same except in Picea smithiana and Cedrus deodara where a surface layer of cells with less number of pericliny is distinct. In E. foliata, the surface layer becomes so discrete as to be called a tunica.

The present studies on the apical organisation of the vegetative buds of the twelve species and the details about other gymnosperms given by earlier workers provide enough data to trace out a few lines of advancement in their shoot apical meristem. (1) The apical initials that are quite distinct in the primitive families like cycadaceae and pinaceae show reduction in size and subsequently disappear as is noticed in Ephedra. (2) The limiting layer of cells of the apices of primitive members undergoes progressive evolution by elimination of periclines to attain the status of tunica in a few members like Ephedra. (3) The fairly large group of central mother cells that are present in the cycads and Ginkgo undergoes reduction in number and

constitutes the specialised subapical initials in some conifers and Ephedrales. (4) The progenitor of pith, the pith rib meristem in cycads, Ginkgo and pines give way to a considerably reduced zone of pith mother cells in Ephedrales.

Seasonal studies on the vegetative buds of two Himalayan conifers, Picea smithiana and Cedrus deodara, show that the annual cycle of these two plants constitutes four phases. A period of dormancy is quite prevalent during the winter. The accumulation of granules of polysaccharides is noticed during the period of dormancy only.

A peculiar anatomical structure called the "crown", reported in some plants by earlier workers but with an unknown function, is noticed only in Picea and Cedrus among all the gymnosperms studied. A thorough investigation of the structure and development of the "crown" provides clues to its possible role in winter dormancy. Specific tests for lignin and pectin are answered positively, thus reaffirming its specialised nature compared to its progenitors, the pith cells. The highly thick-walled cells of the crown retain Feulgen-positive material and protein bodies for nearly one year.

A structure, similar to the crown in gross morphology, but entirely different in details, is noticed in Ephedra foliata. Such a structure, noticed slightly above a node, is characteristic of every mature internode. These two were the regions of the intercalary meristem, that were active along

with the apical meristem to contribute to the primary growth of the vegetative bud. Such an intercalary meristem that retains its specificity for a long time (after its activity) is not reported in any other plant. Further, it is seen that the intercalary growth in E. foliata is not only due to cell multiplication but to cell elongation also. The cells of the pith rib meristem undergo upto 15-fold increase in length with maturation.

Germination studies of the seeds of Picea smithiana show that the cytohistological zonation in the shoot apex becomes as clear as that of the mature plants after 34 days of germination. Further it is seen that the radicular apex and the root apex of the seedling are structurally similar in having a common initiating zone for the stele and columella that is covered by another initiating zone for the cortex and peripheral region of the root cap. The 34-days old seedling shows a root-hypocotyl cotyledon vasculature without any connection with the shoot, where no vasculature has developed.

Studies on the transition of E. foliata from the vegetative to the reproductive show that the tunica-corpus organisation of the vegetative apex is disturbed during bract production in both male and female plants. The apex after bract production can be interpreted by the mantle-core concept. Ontogenic studies of the male strobilus show that there is no indication of a rudiment of the shoot axis, which rules out the possibility of a connation of two microsporophylls, a view suggested by Eames (1952). The patterns of

distribution of total proteins, insoluble polysaccharides, histones, RNA and DNA have been studied in the meristem proper during the transitional phase. Peroxidase localisation during the vegetative and reproductive phases shows greater concentration in the regions of vascular supply and nodal plexus. Acid phosphatase activity, intensely noticed in the corpus initials of the vegetative apex, is shifted to the flank meristem and surface layer cells of the transitional apices.

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PLATE VII

Fig. 1. Median longitudinal section of the shoot apex of Cycas circinalis showing the cytohistological zonation. Staining safranin-fast green.

AI - Apical initials
FM - Flank meristem
CMC - Central mother cells zone
RM - Rib meristem

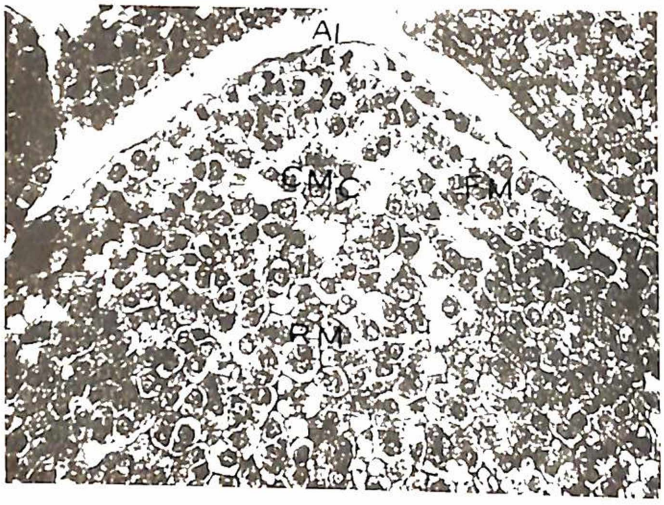
(×180)

Fig. 2. L.S. of the shoot apex of C. circinalis where groups of compactly arranged cells are seen in the CMC zone. Also note the junction belt of cells arranged transversely below the zone of CMC.

(×148)

Figs. 3 and 4. Localisation of insoluble polysaccharides achieved by periodic acid - Schiff's reagent staining. L.S. of C. circinalis apex shows the minimum accumulation of sugars in the CMC zone and maximum in the pith rib meristem.

Fig. 3 (×48)
Fig. 4 (×128)



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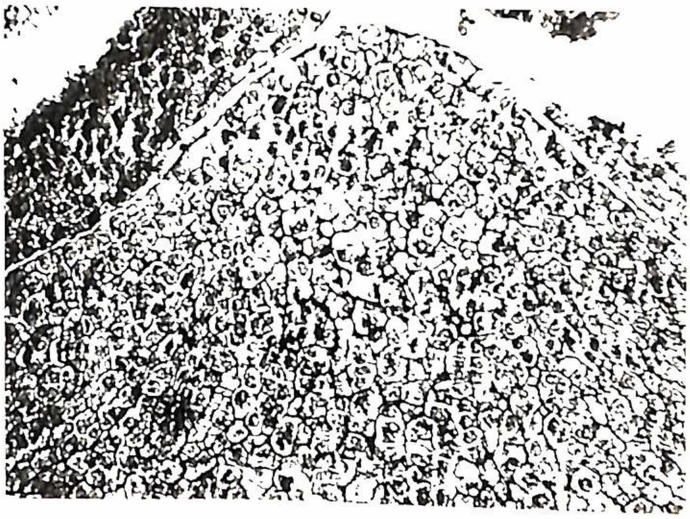

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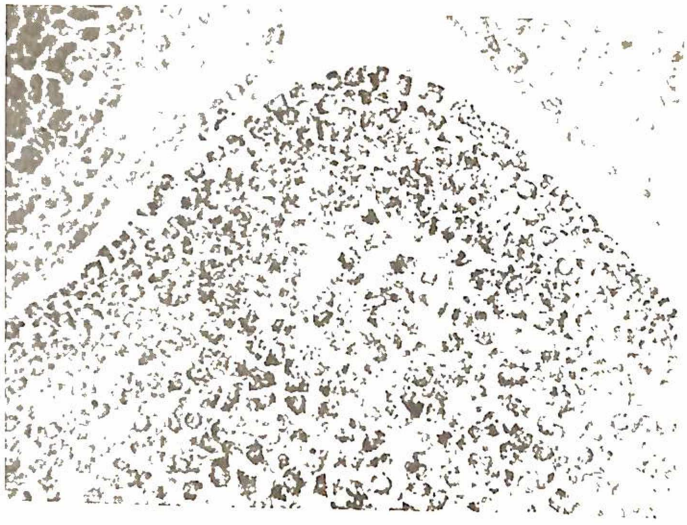

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PLATE. VII

PLATE VIII

Figs. 1 and 2. L.S. of the shoot apices of Pinus roxburghii. Stained with safranin-fast green.

Fig. 1 (×122)
Fig. 2 (×124)

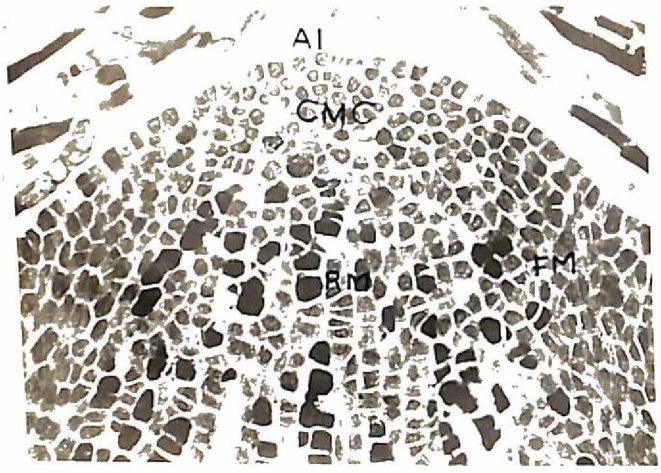
Fig. 3. L.S. of the shoot apex of P. wallichiana. Stained with safranin-fast green, revealing the zonation.

(×160)

Fig. 4. L.S. of the shoot apex of P. insularis. Stained with safranin-fast green.

(×192)

AI - Apical initials
CMC - Central mother cells zone
FM - Flank meristem
RM - Rib meristem



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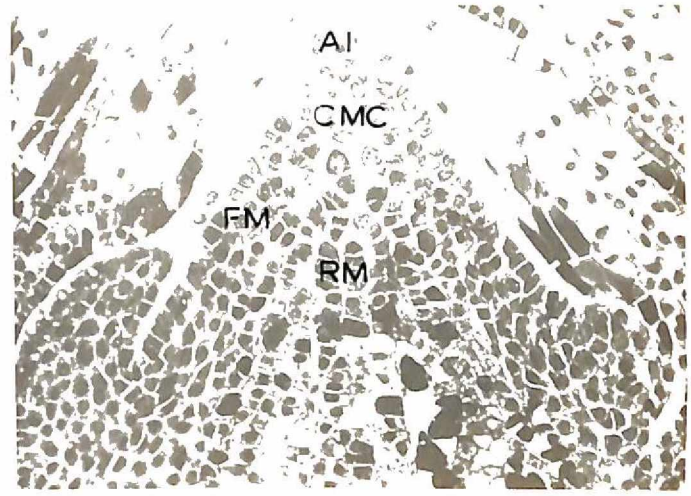

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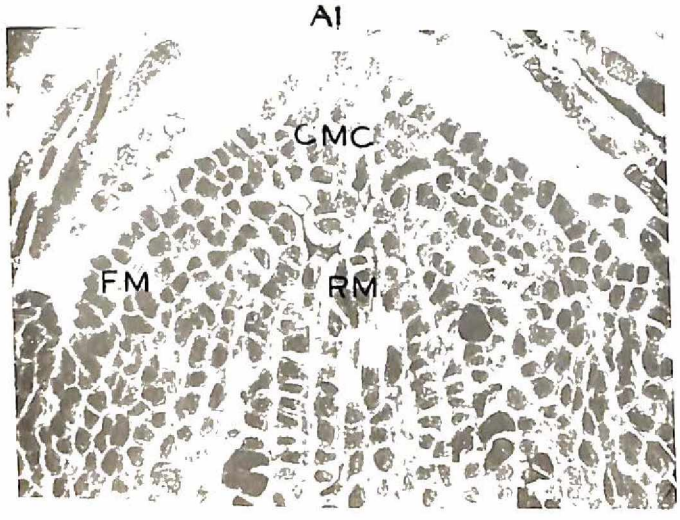

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PLATE VIII

PLATE IX

Fig. 1. L.S. of the shoot apex of Pinus gerardiana. Stained with safranin-fast green. (×192)

Fig. 2. L.S. of the shoot apex of P. pseudostrobus. Stained with safranin-fast green. (×180)

Fig. 3. L.S. of the vegetative apex of P. massoniana. Stained with safranin-fast green. (×180)

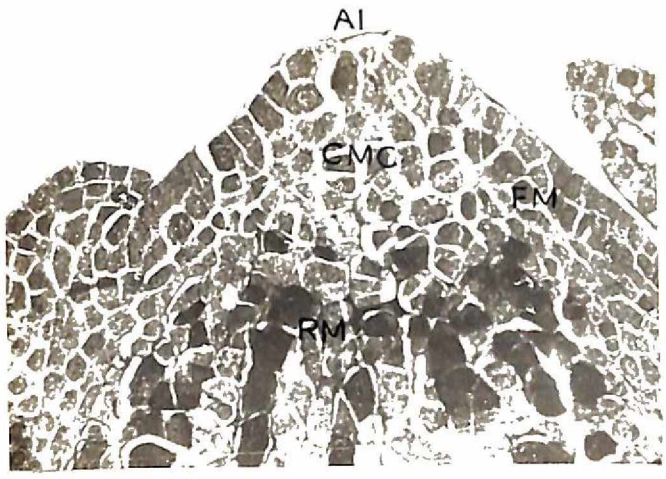
Fig. 4. L.S. of the vegetative apex of P. gerardiana. Stained with safranin-fast green. (×192)

AI - Apical initials

CMC - Central mother cells zone

FM - Flank meristem

PRM - Pith rib meristem



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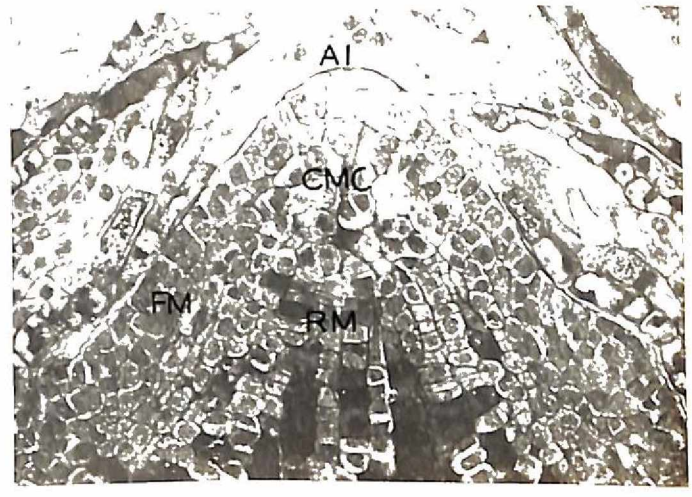

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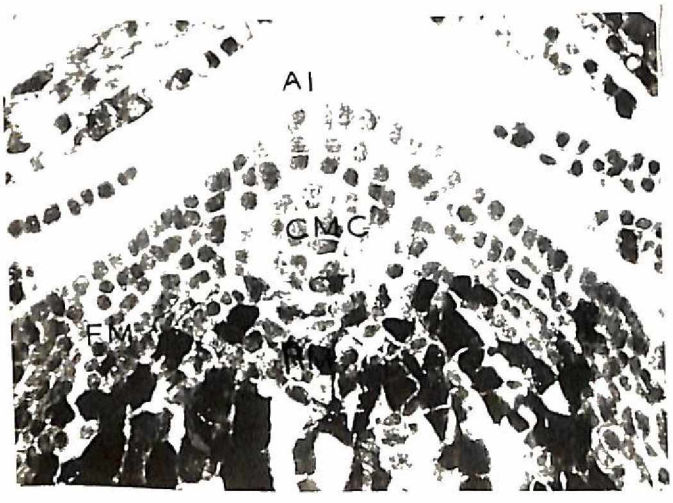

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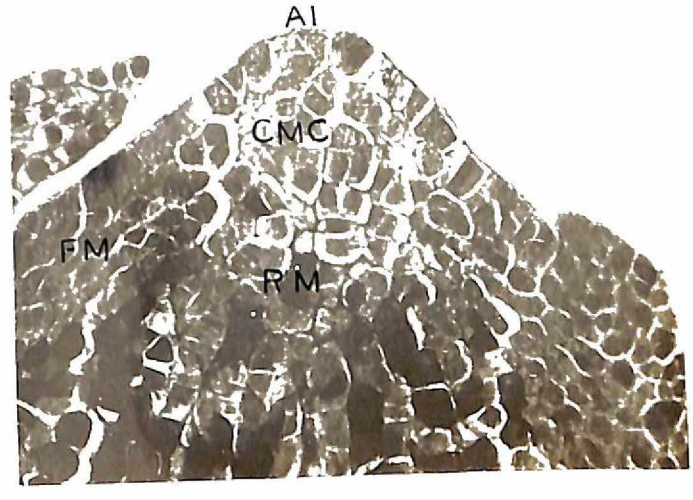

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PLATE IX

PLATE X

Figs. 1 and 2. L.S. of the shoot apices of Abies pindrow stained with safranin-fast green. Zonation is clear in Fig. 1.

Fig. 1 (×236)
Fig. 2 (×243)

Figs. 3 and 4. L.S. of the shoot apices of Taxus baccata stained with safranin-fast green.

Fig. 3 (×144)
Fig. 4 (×130)

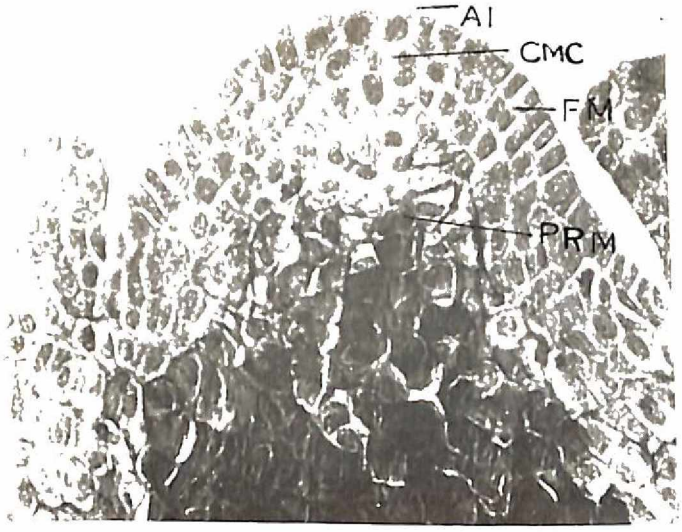
AI - Apical initials

CMC - Central mother cells zone

FM - Flank meristem

PRM - Pith rib meristem

SAI - Subapical initials



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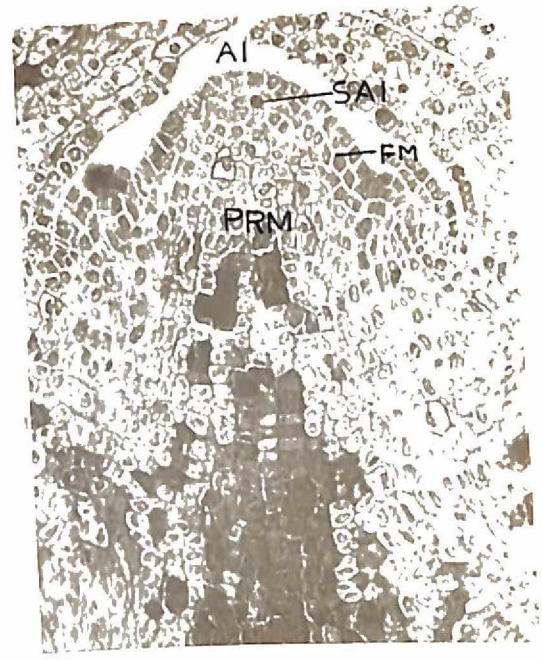

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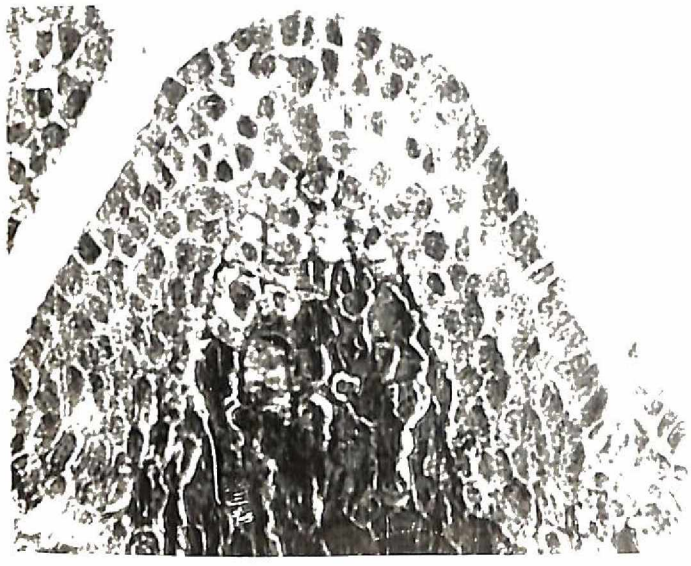

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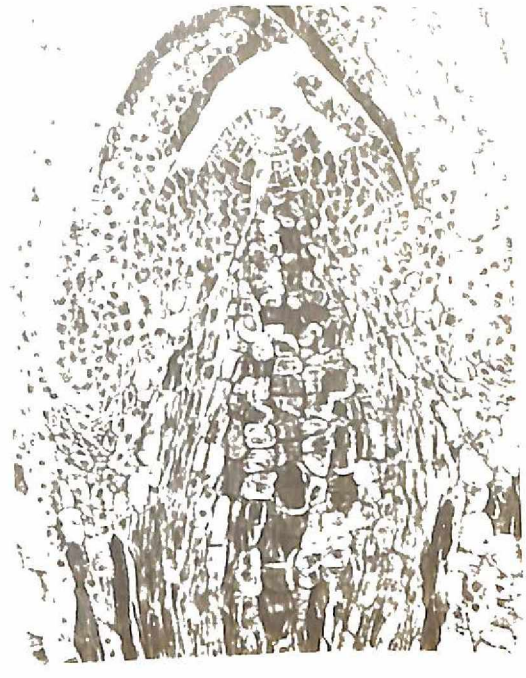

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PLATE X

PLATE XI

Fig. 1. L.S. of the dormant apex of Picea smithiana stained with erythrocine-crystal violet. Collection made during March.
(×220)

Fig. 2. L.S. of the shoot apex of P. smithiana collected during June. Note the periclinal division in the apical initial denoted by the arrow. Staining, safranin-fast green.
(×281)

Fig. 3. Vegetative apex of P. smithiana as seen in longisection stained with safranin fast green. The apex collected in July under extension growth.
(×366)

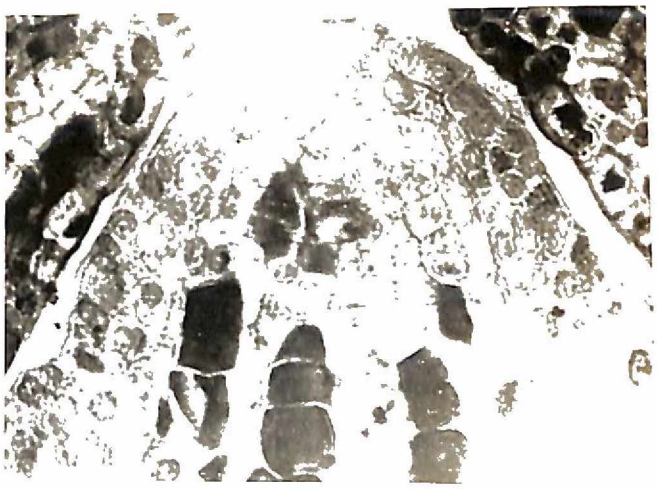
Fig. 4. Apex of P. smithiana collected in the month of August. Stained with periodic acid - Schiff's reagent. Note the zone of sub-apical initials placed partly below the level of the youngest leaf primordia.
(×132)

Fig. 5. The distribution of total proteins as seen in the L.S. of the shoot apex of P. smithiana collected in January, during the period of dormancy.
(×265)

Fig. 6. Longisection of P. smithiana collected during June. Stained for the localisation of total proteins.
(×238)

AI - Apical initials
SAI - Subapical initials
RM - Rib meristem
FM - Flank meristem

1



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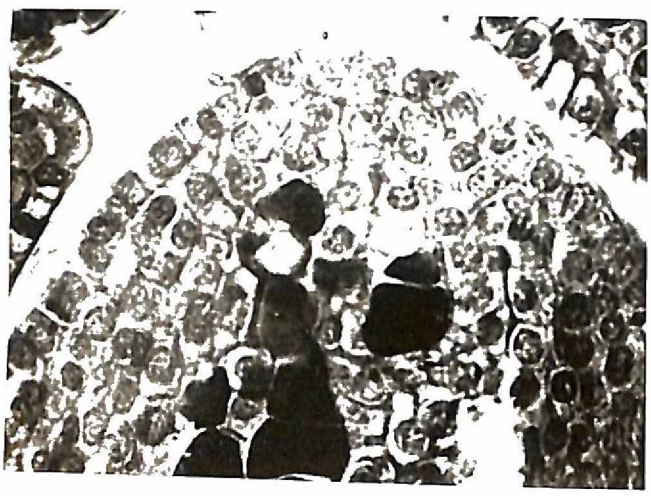

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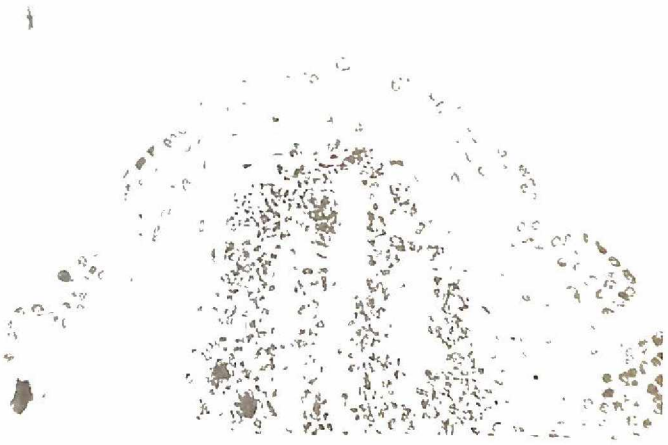

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PLATE XI

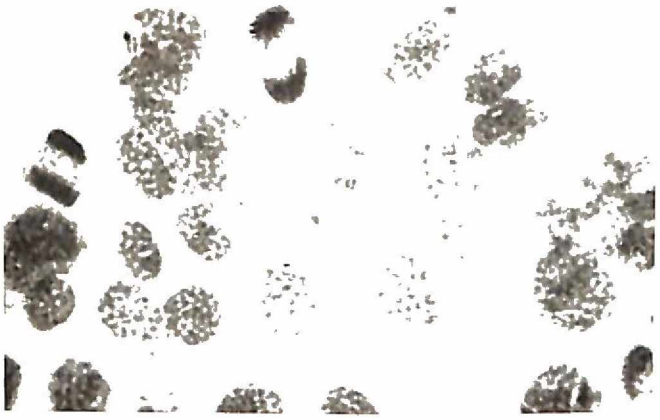
PLATE XII

Figs. 1, 2 and 3. Longisection of vegetative buds of *Picea smithiana* after Feulgen staining. Apices are of April, June and October respectively.

Fig. 1 (×400)

Fig. 2 (×214)

Fig. 3 (×250)



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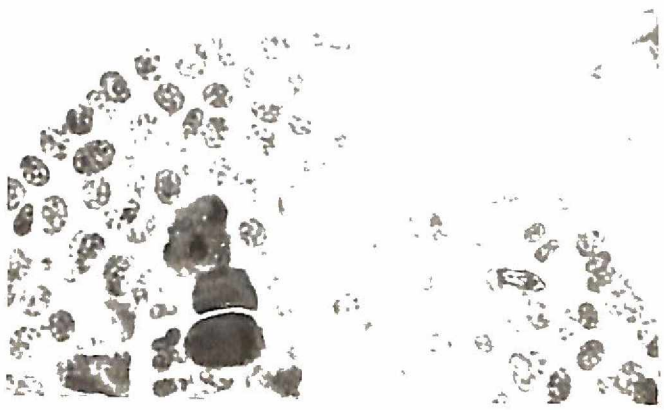

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PLATE XII

PLATE XIII

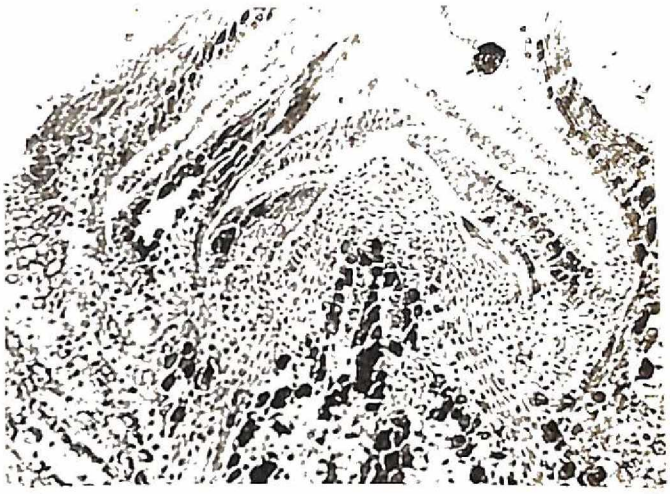
Figs. 1, 2 and 3. Longisections of apices of Picea smithiana stained for insoluble sugars. Note the accumulation of granules of polysaccharides till the subapical initials zone in the February bud (Fig. 1) and its absence in the buds of May and August (Figs. 2 and 3).

Fig. 1 (×190)
Fig. 2 (×156)
Fig. 3 (×270)

Figs. 4, 5 and 6. Vegetative apices of P. smithiana sectioned and stained for histones. Fig. 4 refers to a bud collected in June, 5 to October and 6 to December.

Fig. 4 (×340)
Fig. 5 (×340)
Fig. 6 (×280)

1



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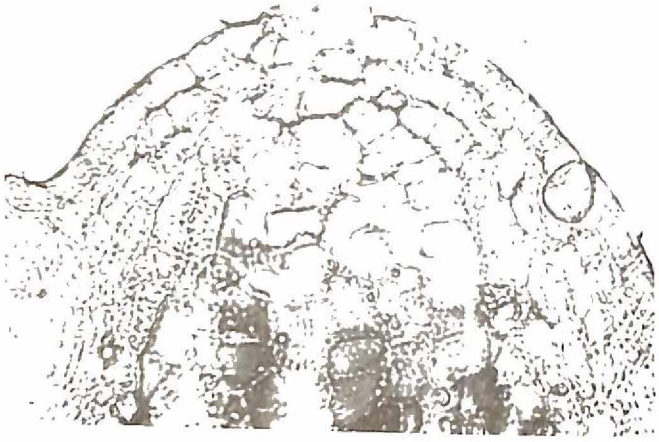

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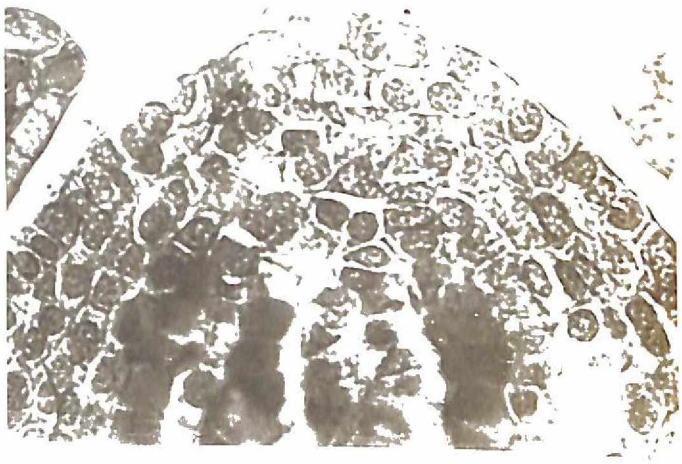

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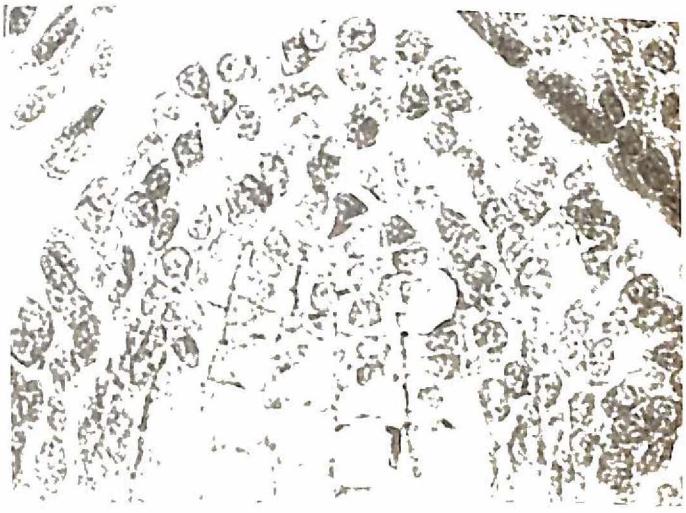

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PLATE XIII

PLATE XIV

Longitudinal sections of vegetative buds of *Cedrus deodara*.

Fig. 1. The median section of a long shoot collected in May, showing the cytohistological zonation. Note the comparatively bigger cells of apical initials and the arrow showing a pericline. Staining safranin-fast green.

(×300)

Fig. 2. The dwarf shoot of *C. deodara*, as seen in a longisection stained with safranin-fast green The crown is denoted with the letter CR.

(×108)

Fig. 3. A longisection of a bud collected during September, and stained with safranin fast green.

(×272)

Fig. 4. The long shoot during the bud elongation period. The apex was collected in the month of April and stained with periodic acid Schiff's reagent.

(×40)

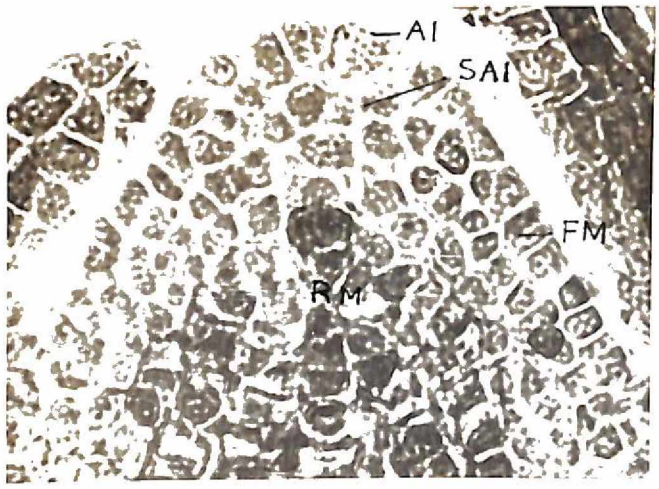
Fig. 5. An apex collected during March and stained with safranin-fast green.

(×300)

Fig. 6. An apex collected in the month of July. Note that the shoot apex is located in a depression formed by the continued production of cataphylls.

(×82)

AI - Apical initials
SAI - Subapical initials
RM - Rib meristem
FM - Flank meristem



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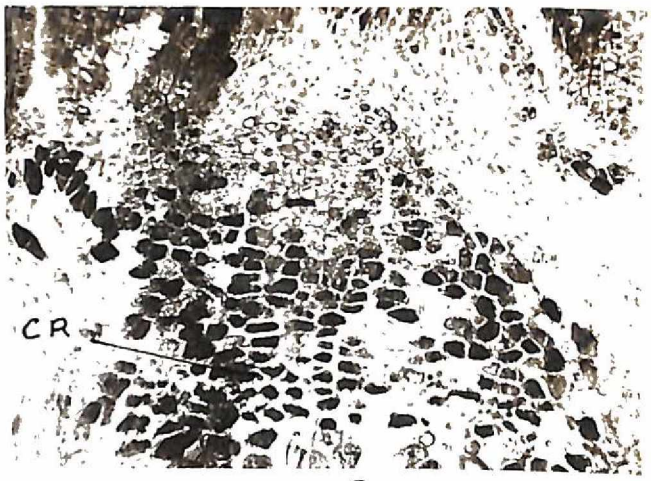

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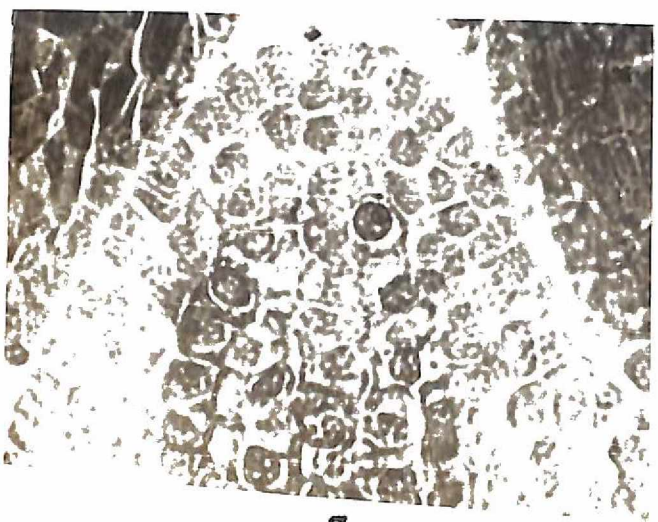

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PLATE XIV

PLATE XV

Median longitudinal sections of shoot apices of Cedrus deodara that are Feulgen stained to reveal DNA.

Fig. 1. A dormant bud collected in January. (×320)

Fig. 2. An active bud of March. Note the divisional phases in the surface layer cells. (×300)

Fig. 3. A predominant bud collected in the month of October. Note the uniform stainability of the nuclei irrespective of the zone. (×235)

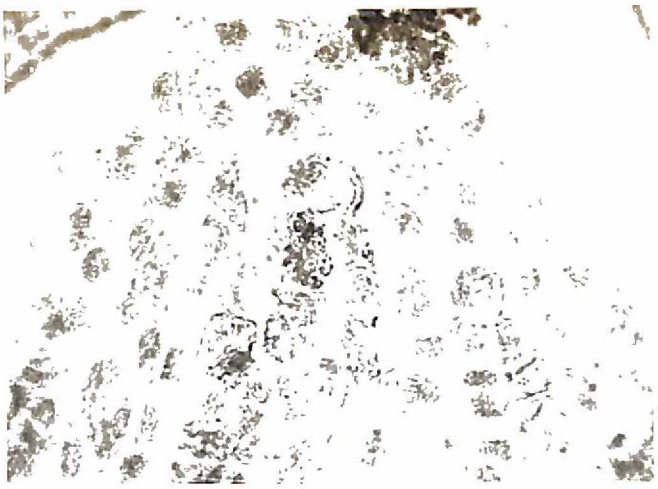

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PLATE XV

PLATE XVI

Fig. 1. A vegetative bud of Cedrus deodara collected in September. Granules of polysaccharides are noticed in the rib meristem of the PAS. Stained longisection.

(×240)

Fig. 2. An overwintering bud of C. deodara collected in April, stained to reveal insoluble carbohydrates. Starch granules are completely absent.

(×260)

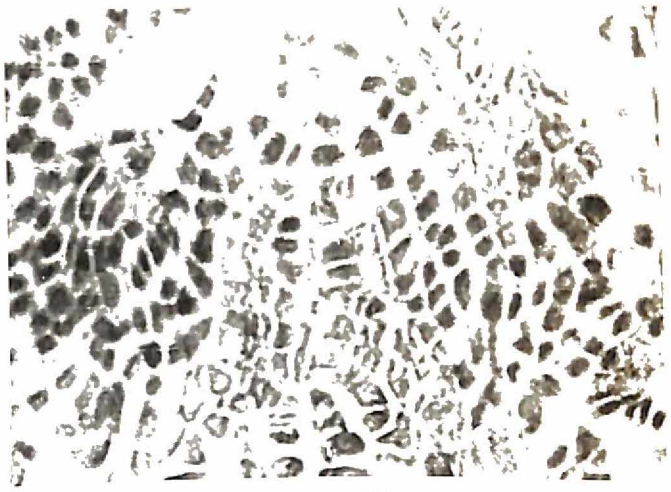
Figs. 3 and 4. Median longisections of shoot apices of C. deodara stained for total proteins. Fig. 3 refers to the apex collected in October and 4 to one collected in July.

Fig. 3 (×302)
Fig. 4 (×252)

Fig. 5. An apex of C. deodara collected in the month of July, sectioned longitudinally and stained for histones. Note the stainability of the cytoplasm.

(×400)

1



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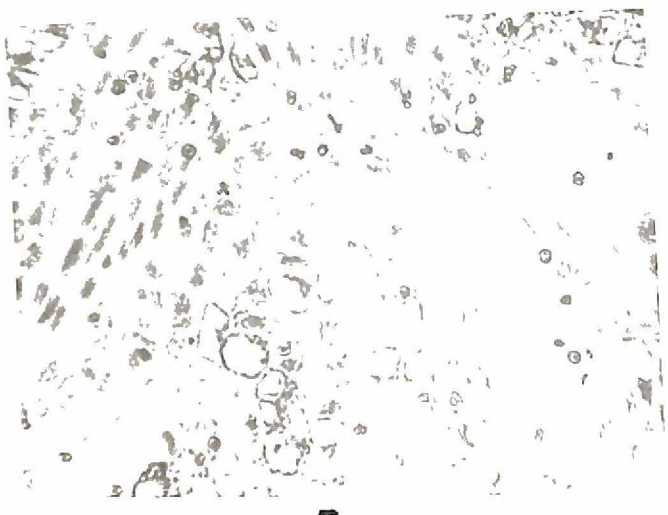

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PLATE XVI

PLATE XVII

Fig. 1. L.S. of the dormant bud of Picea smithiana collected in the month of January. The crown is distinct with the thick walled cells arranged in 3-4 layers. Also note the accumulation of some coloured contents in the pith above the crown. Staining with PAS.

(×38)

Fig. 2. The dormant bud of Cedrus deodara as seen in a longisection stained with PAS. The crown cells and the pith cells above the crown are rich in starch granules.

(×65)

Fig. 3. The region of crown in a dormant bud of Picea smithiana, showing the distribution of polysaccharides, revealed by PAS staining.

(×90)

Fig. 4. A magnified view of the crown region as seen in a longisection of C. deodara, stained with safranin-fast green. Note the distinct nuclei in the crown cells compared to the disorganised cells of the region below.

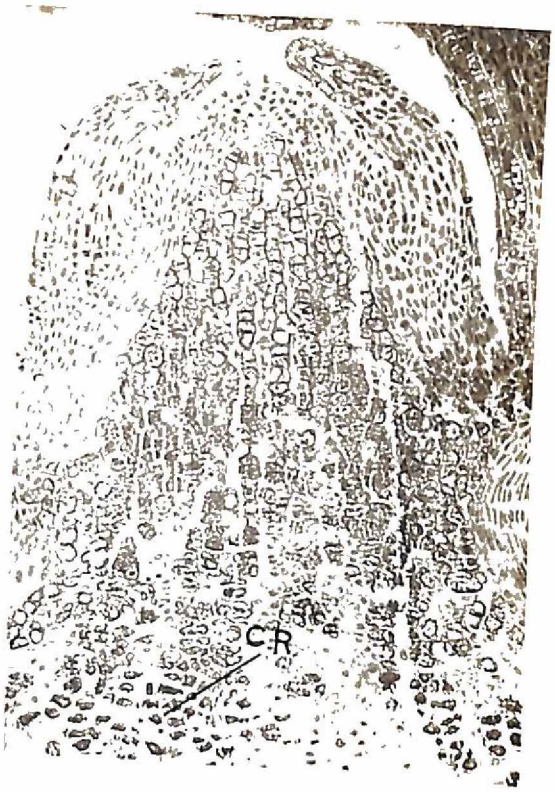
(×844)

Fig. 5. The lignin localisation in the crown cells, revealed by phloroglucinol test. The material collected in April from C. deodara is more than one year old.

(×60)

CR - Crown

1



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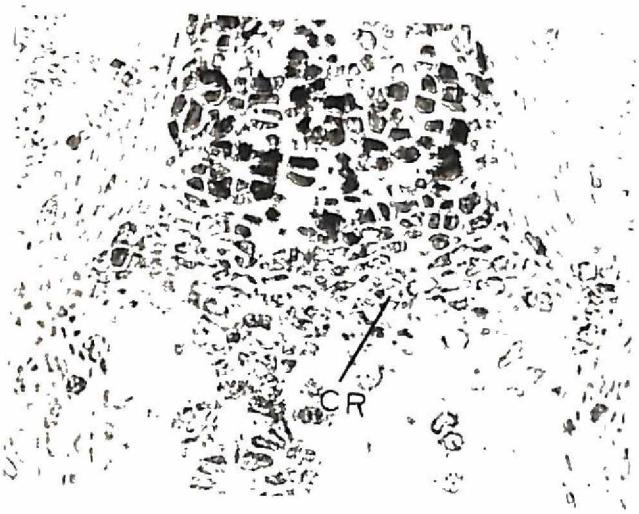

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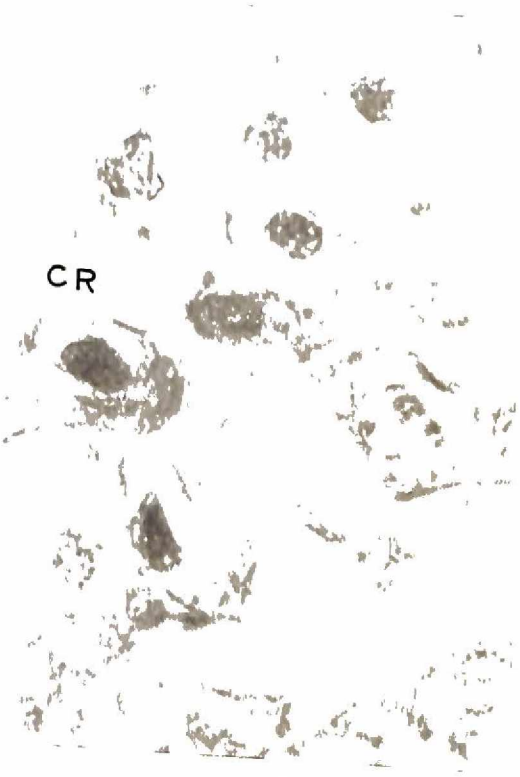

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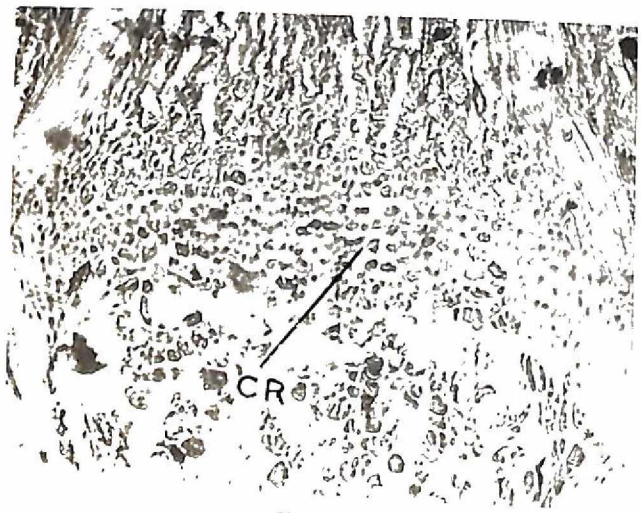

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PLATE XVII

PLATE XVIII

Fig. 1. Feulgen stained longisection of the dormant bud of Picea smithiana.

(× 38)

Fig. 2. L.S. of the dwarf shoot of Cedrus deodara stained stained witn safranin-fast green. Two crowns are distinct. The distance between the new and old crowns denotes one year's growth of the bud.

(×30)

Fig. 3. L.S. of the vegetative bud of P. smithiana collected in October and stained with safranin fast green. The initiation of crown is noticed with transverse elongation of some pith cells.

(× 112)

Fig. 4. L.S. of the vegetative buds of P. smithiana collected in January. Safranin-fast green staining makes the thick walled cells of the crown quite distinct.

(× 140)

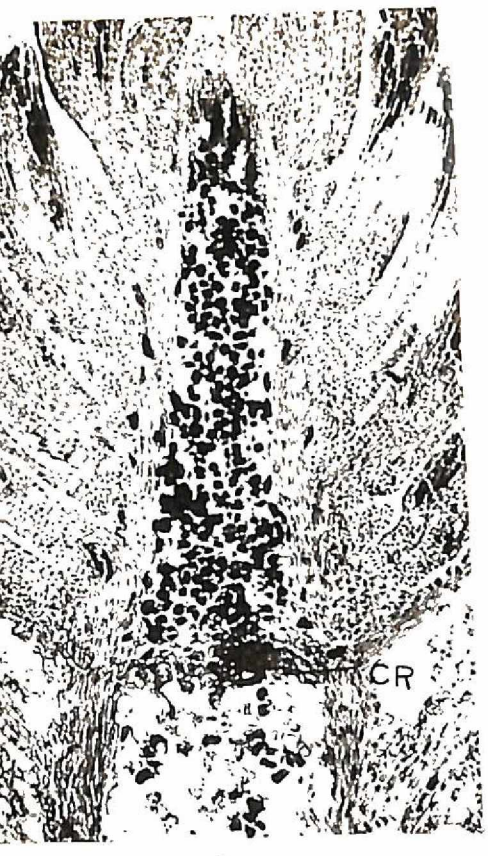
Fig. 5. An overwintering bud of P. smithiana as seen in a longisection stained with erythrocine violet. Note the distinct nuclei of crown cells and the newly formed cell lineage just above the crown. Also note the disorganised pith cells below the crown.

(× 98)

Fig. 6. L.S. of the vegetative bud of P. smithiana during the period of bud elongation. Note the development of vascular elements above the level of crown. Staining safranin-fast green.

(×102)

1st CR - First crown
2nd CR - Second crown
CR - Crown



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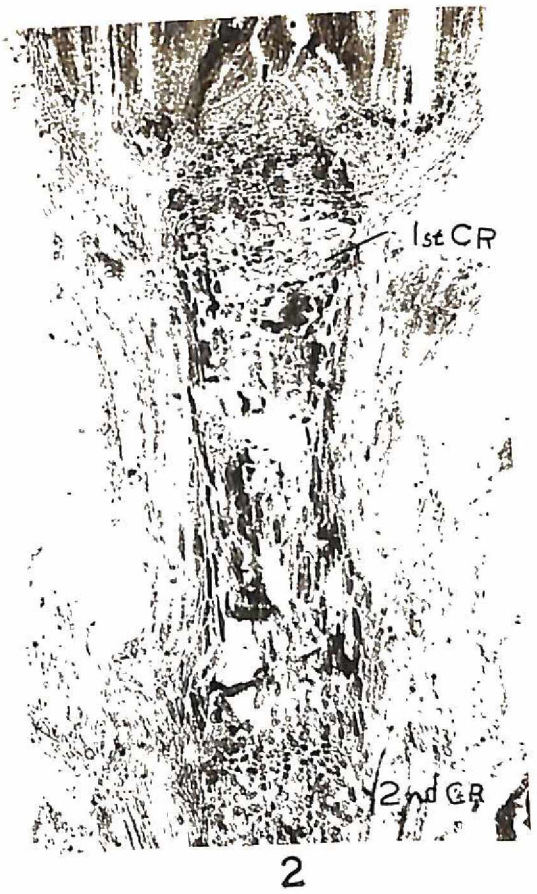

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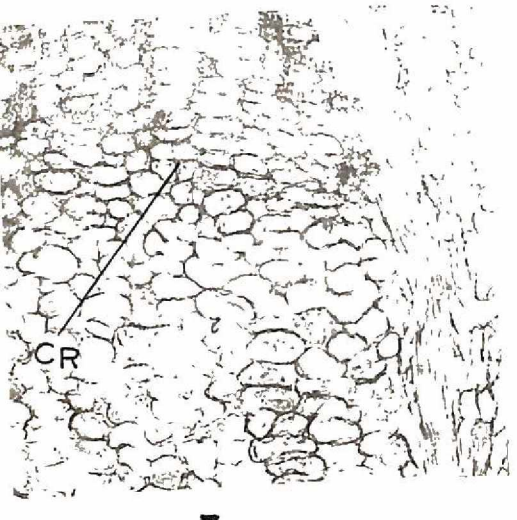

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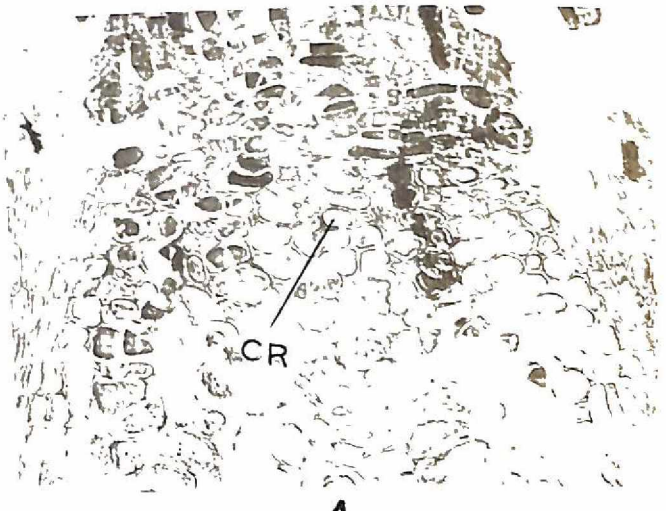

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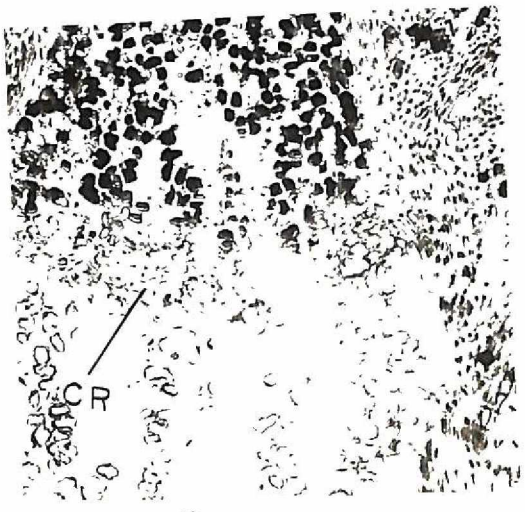

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PLATE XVIII

PLATE XIX

Intercalary meristem in *Ephedra foliata*

Fig. 1. Median longitudinal section of the 4th internode from the shoot tip after Feulgen staining.

(× 428)

Fig. 2. The region of intercalary meristem as seen in a L.S. of the 6th internode. Note the ill-defined boundary of the region slightly above the 7th node.

(× 100)

Fig. 3. The region of intercalary meristem in the 8th node revealed by safranin-fast green staining.

(× 80)

Fig. 4. Portion of the intercalary meristem region of 5th internode. Note the less vascuolate cells of the intercalary meristem compared to the highly vacuolated cells above. Staining safranin-fast green.

(× 510)

Fig. 5. A comparatively old internode with most of the pith cells pitted. Note the transverse belt of 3-4 cells that are thin walled and transversely elongated, above the nodal plexus. Erythrocine crystal violet staining.

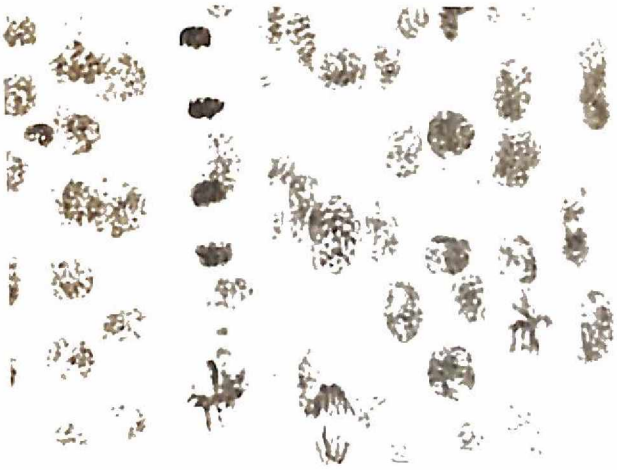
(× 95)

Fig. 6, L.S. of an approximately 4 year old stem at the level of the intercalary meristem. The nucleate and thin walled cells of the belt are quite distinct against the anucleate and highly pitted cells of the pith. The xylem elements are seen touching these cells.

(× 282)

IM - Intercalary meristem

NP - Nodal plate



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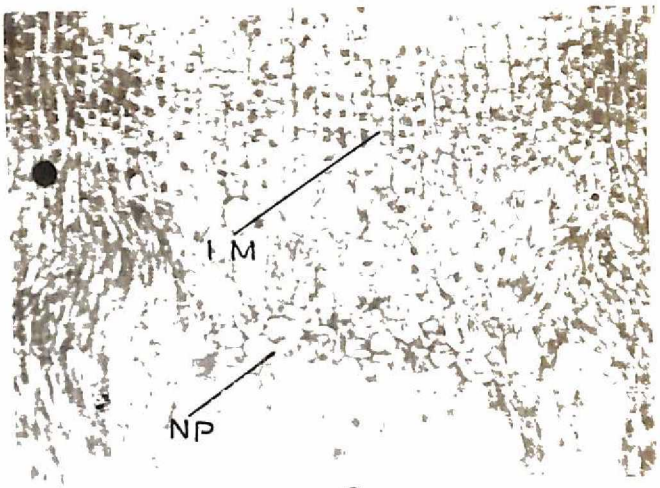

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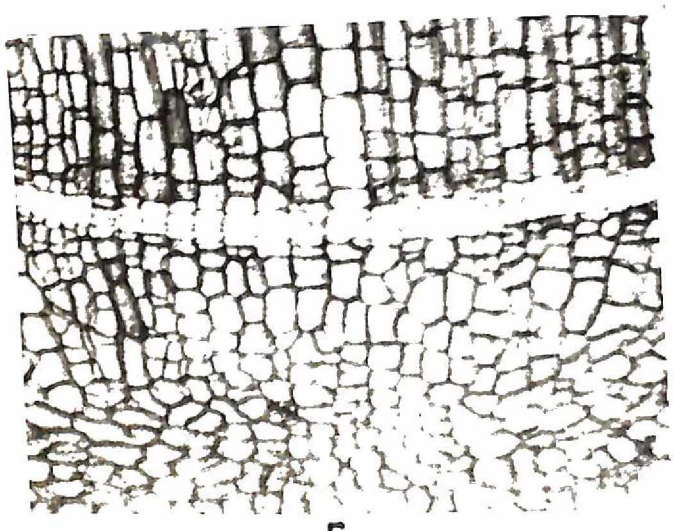

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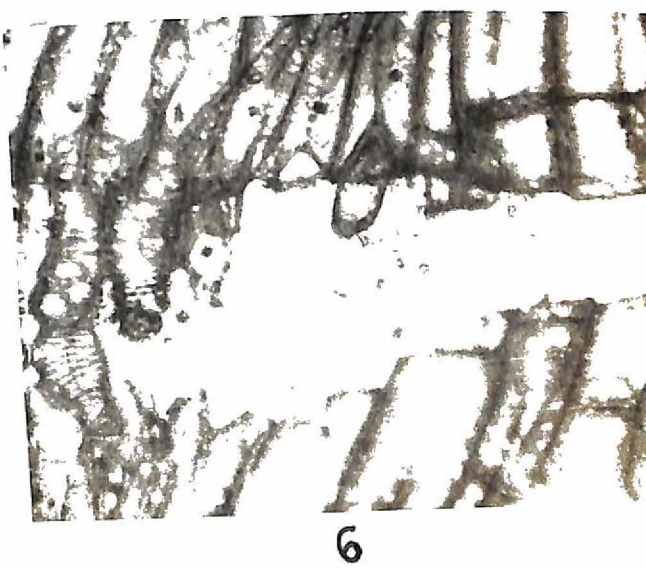

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PLATE XIX

PLATE XX

Fig. 1. Seedlings of Picea smithiana after 45, 34, 25, 15 and 10 days after germination.

Fig. 2. Median longisection of shoot apex of mature embryo one hour after seed wetting. Safranin fast green staining. Arrow indicates periclinal division.

(× 510)

Fig. 3. Median L.S. of shoot apex of P. smithiana after 34 days of germination.

(× 165)

Fig. 4. Median L.S. of shoot apex of 45 day old seedling.

(× 322)

Fig. 5. Median L.S. of radicular apex of mature embryo.

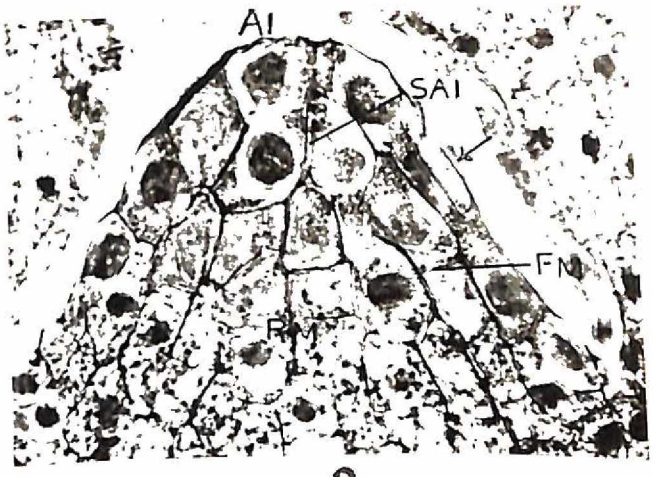
(× 165)

Fig. 6. Median L.S. of root apex of 34 day old seedling.

(× 205)

AI - Apical initials
SAI - Subapical initials
SL - Surface layer on the flanks
F - Flanking zone
RM - Rib meristem
NP - Needle primordium
S - Stele
RI - Root initials
C - Columella
CI - Cortical initials

1



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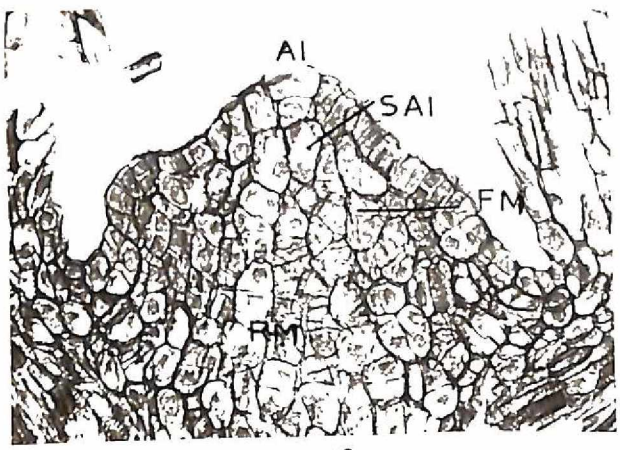

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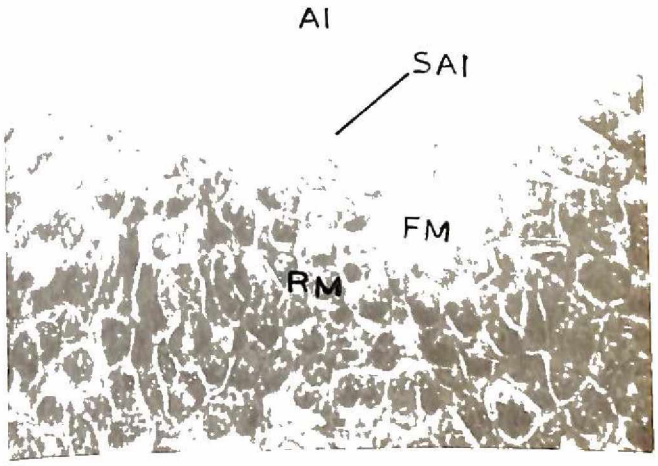

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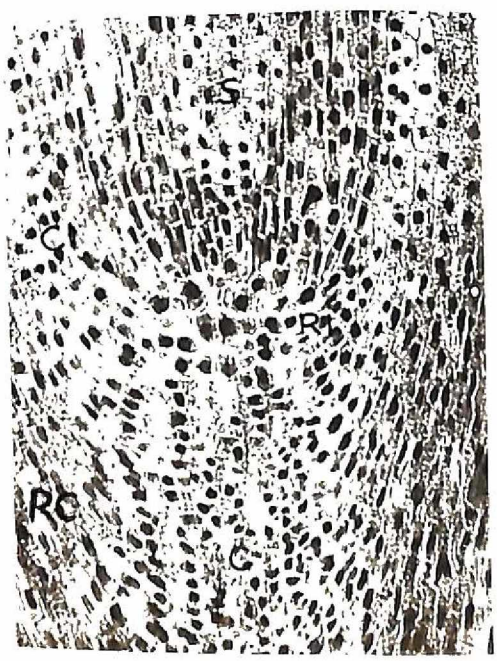

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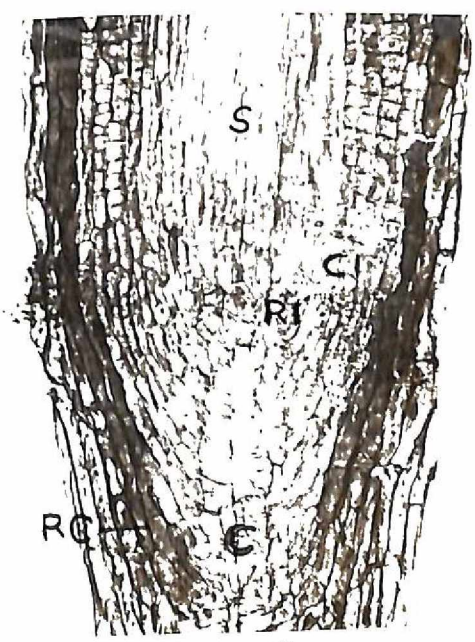

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PLATE XX

PLATE XXI

Fig. 1. Transverse section of root of seedling 2050 μ above the root initials.

(× 205)

Fig. 2. T.S. of root of seedling 4500 μ above root initials.

(× 160)

Fig. 3. T.S. of hypocotyl of 25 μ below shoot initials.

(× 205)

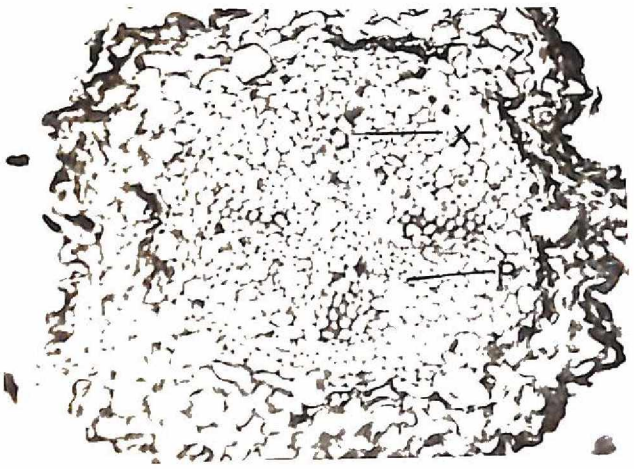
Fig. 4. T.S. of portion of hypocotyl.

(× 360)

[illegible] (× 205)

[illegible] just below the ground [illegible] vascular traces. (× 160)

x - Xylem
P - Phloem
PP = Precursory phloem



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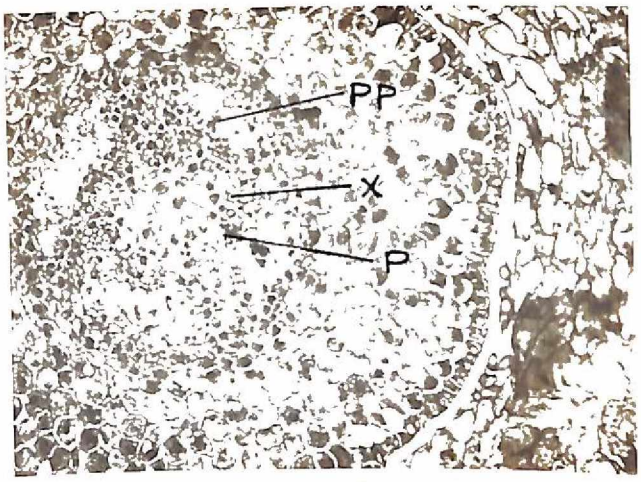

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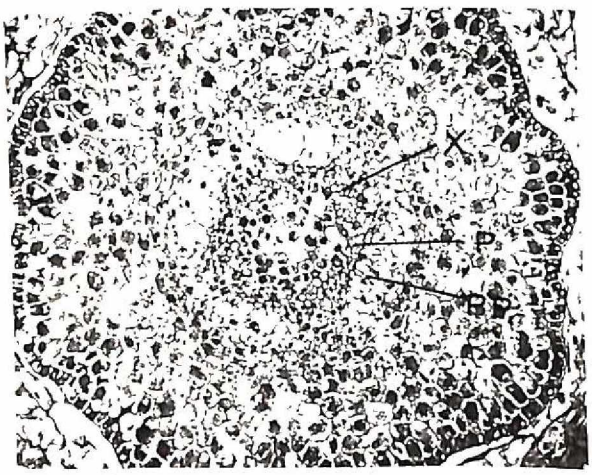

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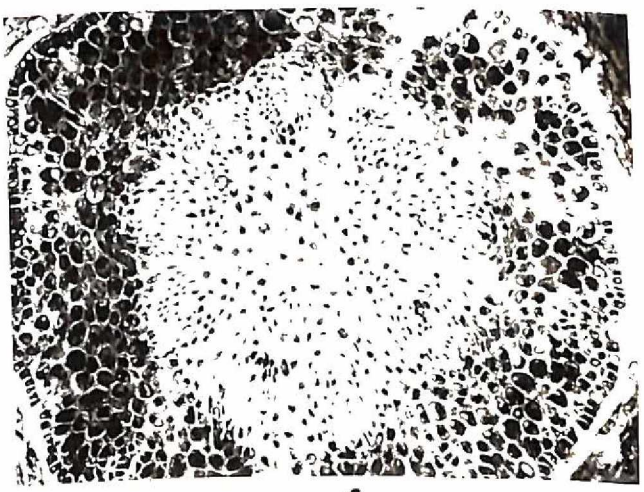

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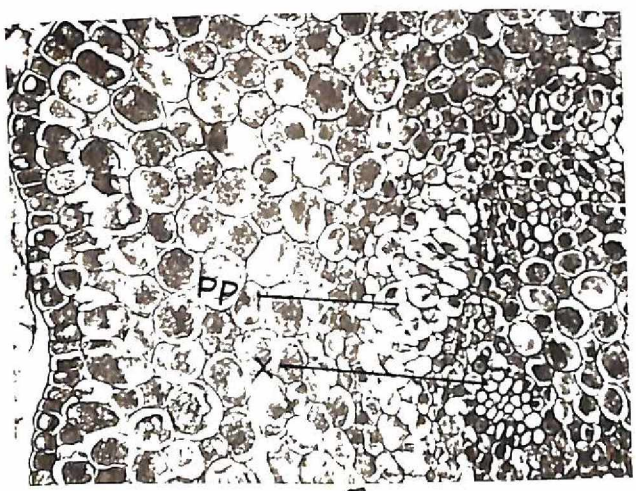

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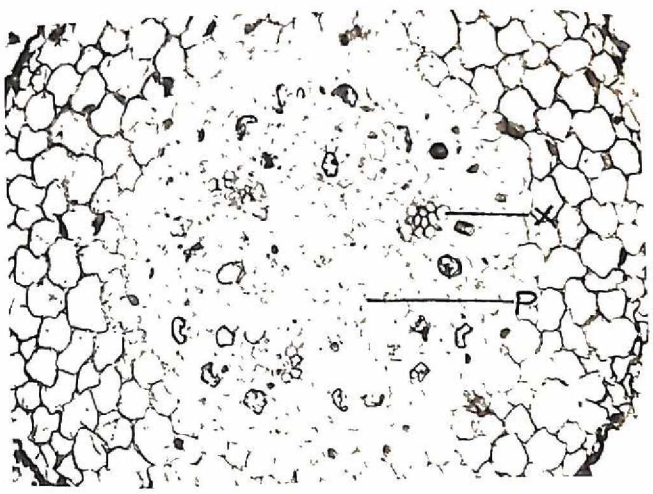

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PLATE XXI

PLATE XXII

Phase shift of Ephedra foliata shoot apices from vegetative to reproductive

Fig. 1. The aerial portion of a female plant bearing cones.

Fig. 2. L.S. of the vegetative apex of female plant stained with safranin fast green.

(× 340)

Fig. 3. L.S. of the apex of a female plant during the middle of bract production. Staining safranin fast green.

(× 252)

Fig. 4. L.S. of the apex during the final stages of bract production. The region showing pericline is marked by arrow.

(× 196)

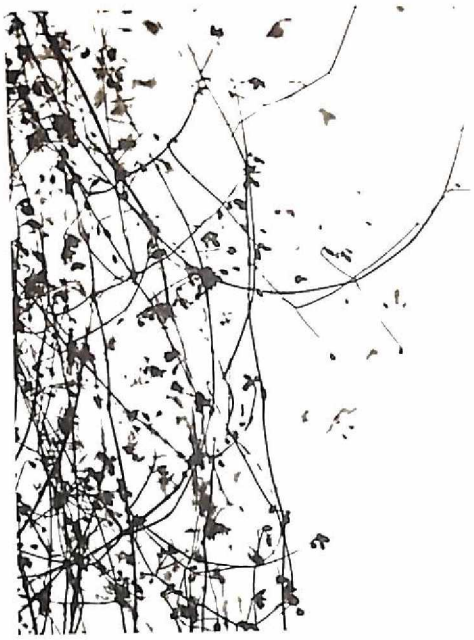
Fig. 5. The apex of the female plant after bract production. Safranin fast green staining.

(× 315)

Fig. 6. L.S. of the transitional apex of a female plant stained with PAS. Note the polarisation of activity at the two distal poles of the dome.

(× 170)

CI - Corpus initials
T - Tunica
FM - Flank meristem
PMC - Pith mother cells



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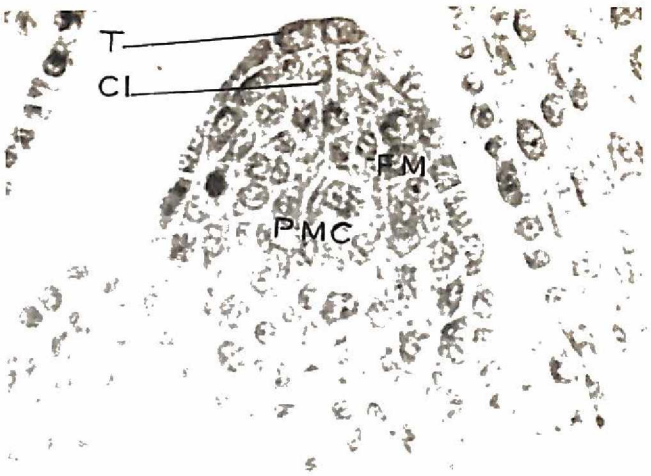

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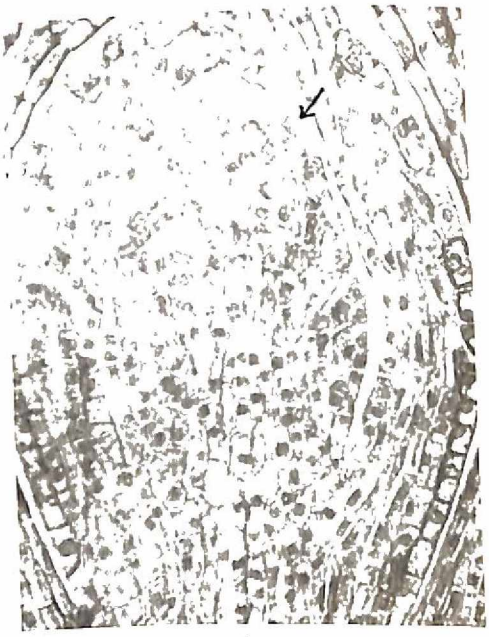

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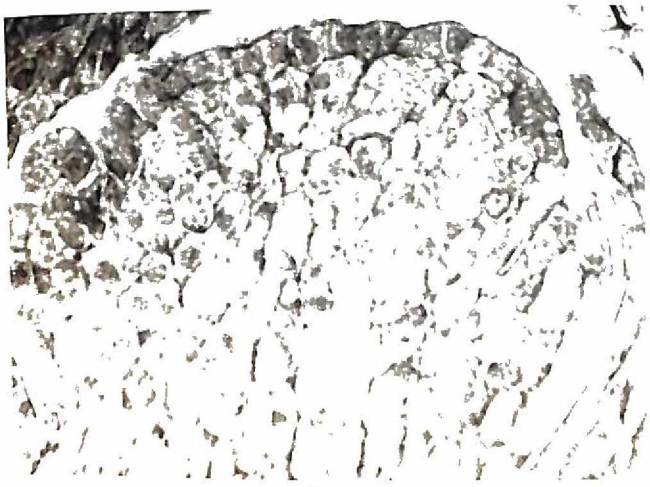

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6

PLATE XXII

PLATE XXIII

Phase shift of *Ephedra foliata* shoot apices from vegetative to reproductive (contd.)

Fig. 1. L.S. of the transitional apex of a female plant stained with periodic acid Shiff's reagent. Note the polarisation of activity at the two distal poles of the dome.

(× 171)

Fig. 2. L.S. of a female cone during the perianth production. Arrow denotes the peridine on the surface layer. Staining safranin fast green.

(× 282)

Figs. 3 and 4. Ovulate strobili after perianth production. Arrow indicates the region of periclinal division.

Fig.3(× 179)
Fig.4(× 238)

Fig. 5. L.S. of a vegetative apex collected from a male plant, Stained with safranin fast green.

(× 315)

Fig. 6. A bract producing apex collected from a male plant.

(× 327)

FP - Floral primordium
T - Tunica
CI - Corpus initials
PMC - Pith mother cells
FM - Flank meristem

PLATE XXIV

Phase shift of *Ephedra foliata* shoot apices from vegetative to reproductive (contd.)

Fig. 1. A longisection of a male cone showing the apex and lateral primordia. Note the greater stainability and increase in size of 2-3 subsurface layer cells of the floral primordium.

(× 327)

Fig. 2. L.S. of the male cone showing the floral primordium as well as bract primordium. Note the precocious development of the anterior lobe of the bracteole. Staining safranin fast green. (BR - Bract)

(× 292)

Fig. 3. L.S. of a young male flower after safranin fast green staining.

(× 326)

Fig. 4. An antherophore bearing the sporangia. Staining used is PAS.

(× 128)

Figs. 5, 6 and 7. Apices stained to reveal distribution of total proteins.

5. L.S. of a vegetative apex stained with bromophenol blue to reveal total proteins. The black spots denote the nucleoli.

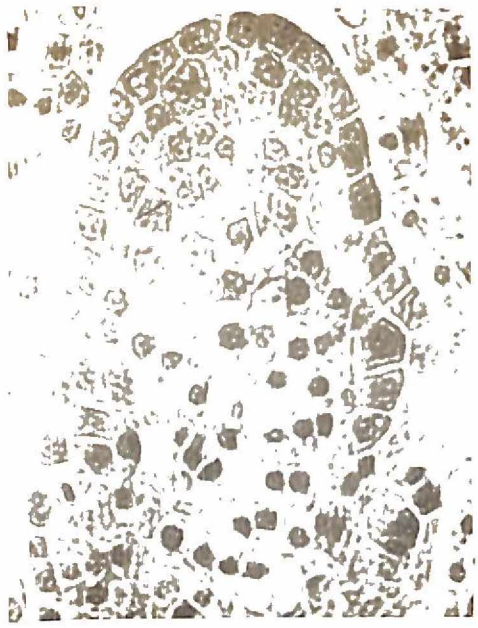
(× 326)

6. The floral meristem in a female cone flanked by the integuments.

(× 310)

7. L.S. of a male cone.

(× 352)



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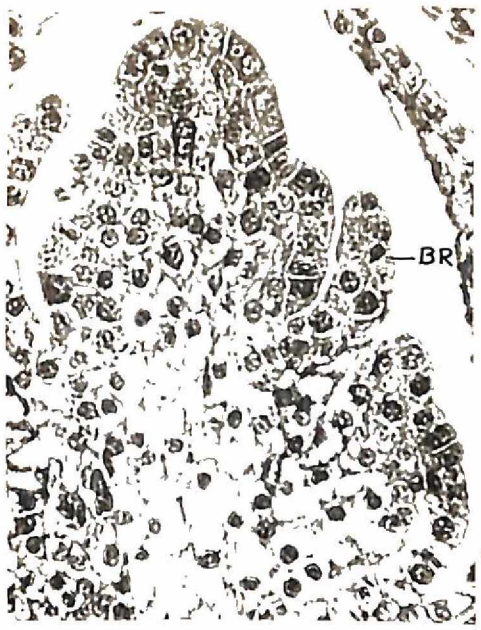

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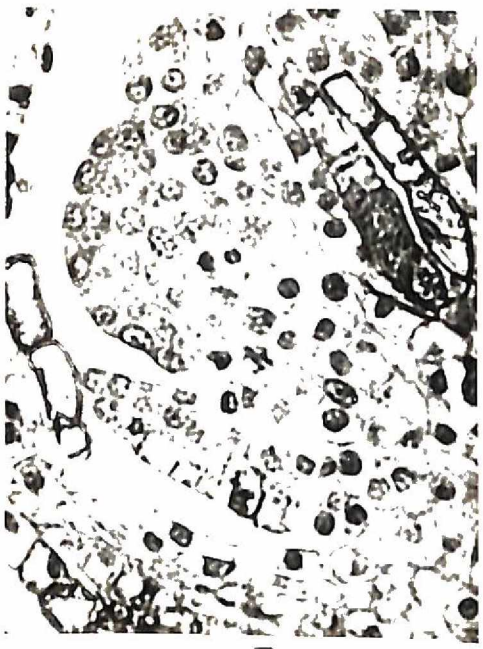

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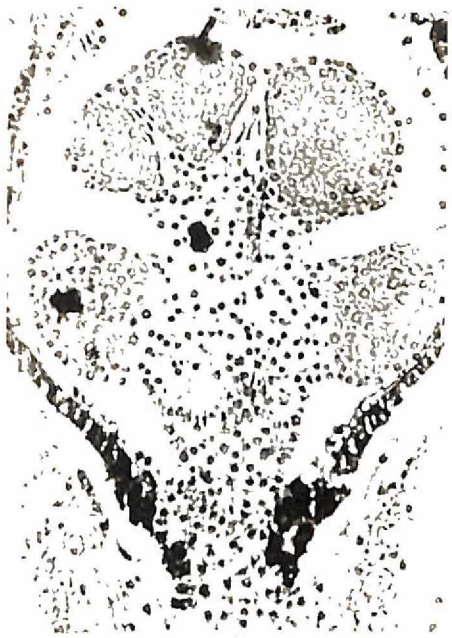

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PLATE XXIV

Plate XVI

Photomicrographs of shoot apices of Ephedra foliata from vegetative to reproductive condition. (Iyer & Maheshwari)

Fig. 1. L.S. of a bract producing apex collected from a female plant.

(× 240)

Fig. 2. L.S. of a young male cone.

(× 370)

Fig. 3. L.S. of a male cone showing the floral primordia. Note the large nucleoli of the cells.

(× 197)

Fig. 4. L.S. of a young male flower before elongation of the antherophore. Note the difference in nuclear details of the outer and inner cells.

(× 430)

Fig. 5. L.S. of a male cone. Note the greater stainability of the surface cells as well as the cells disposed in the humps.

(× 348)

Fig. 6. A female cone with two female flowers.

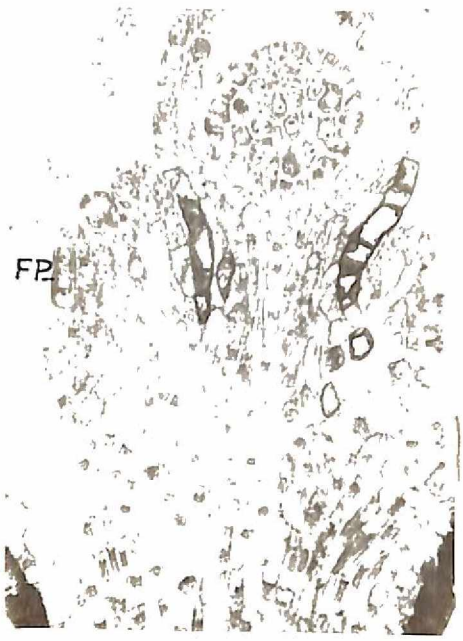
(× 292)

FP - Floral primordium

NO - Nucleolus

1

2



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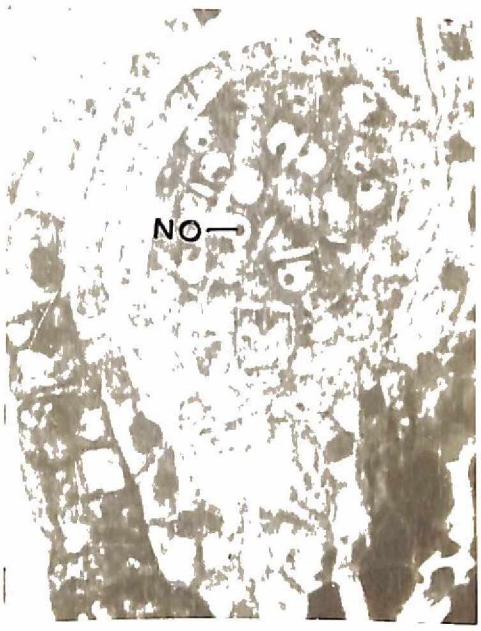

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PLATE XXV

PLATE XXVI

Phase shift of *Ephedra foliata* shoot apices from vegetative to reproductive (contd.) Feulgen staining

Fig. 1. A vegetative apex in L.S.

(× 913)

Fig. 2. The transforming apex of a female plant during bract production. Note the two divisional figures in the surface layer.

(× 320)

Fig. 3. The transforming apex of a female plant after bract production. Note the layering of the outer nuclei.

(× 340)

Fig. 4. The flower initiation in a strobilar apex. Note the disturbance of the layering due to localised activity.

(× 390)

Figs. 5 and 6. Longisections of a young male cone and a flower.

Fig. 5 (× 322)

Fig. 6 (× 378)

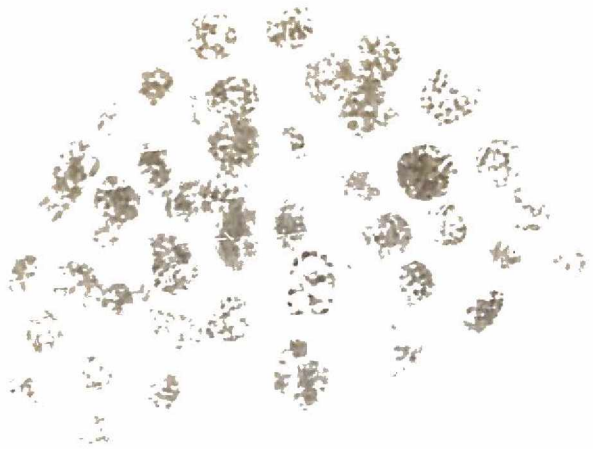

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PLATE XXVI

PLATE XXVII

Phase shift of *Ephedra foliata* shoot apices from vegetative to reproductive (contd.). Fast green FCF staining revealing histone localisation.

Fig. 1. L.S. of a vegetative apex.

(× 532)

Fig. 2. Magnified view of a bract producing apex. Note the divisional phase of a corner cell.

(× 1536)

Fig. 3. The apex of a female plant during bract production.

(× 378)

Fig. 4. L.S. of the apex of a female plant after bract production. Note the uniform staining of the dome.

(× 416)

Fig. 5. The globose head of an antherophore primordium showing the uniform stainability.

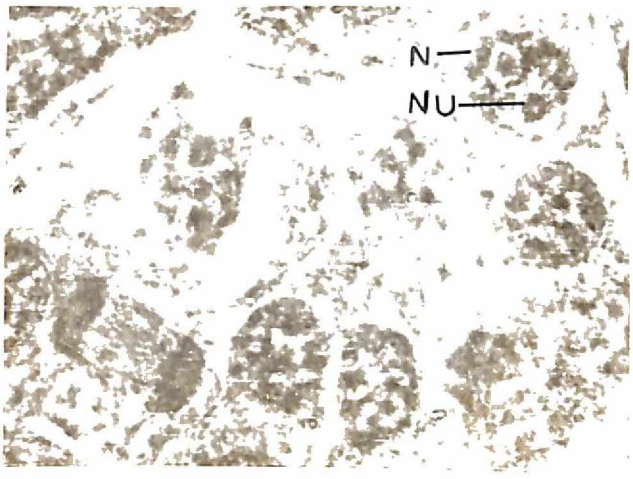
(× 340)

Fig. 6. L.S. of the apex of a female cone after the production of outer integument.

(× 262)

NU - Nucleolus
N - Nucleus
BP - Bract primordium
FP - Floral primordium

1



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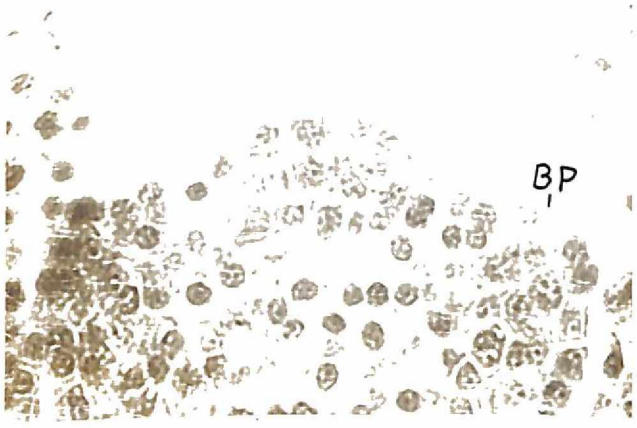

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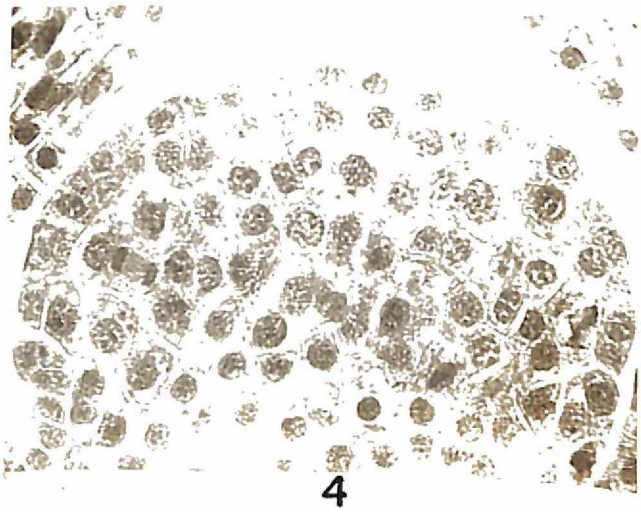

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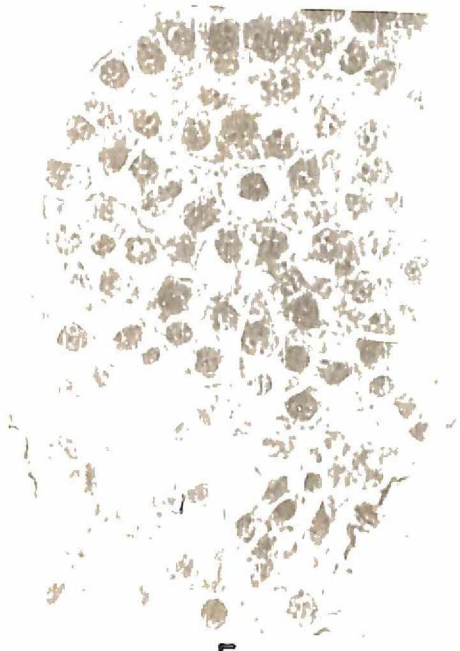

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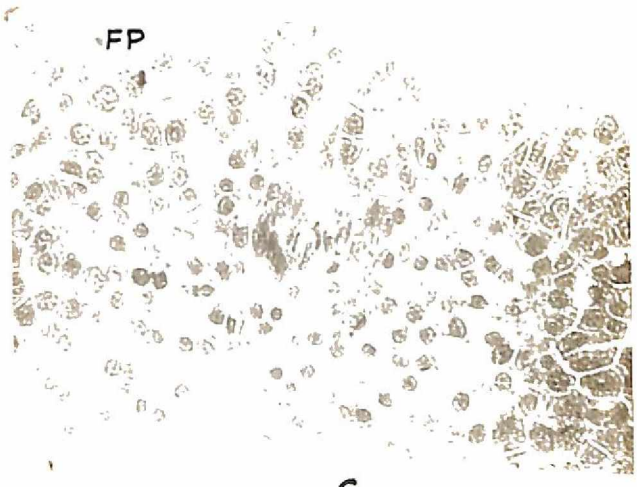

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PLATE XXVII

PLATE XXVIII

Phase shift of *Ephedra foliata* shoot apices from vegetative to reproductive (contd.). Enzyme localisation.

Fig. 1. Longisection of the vegetative apex stained for peroxidase localisation.

(× 120)

Fig. 2. A female floral primordium sectioned longitudinally and stained for peroxidase. Note the greater concentration of the enzyme in the region of attachment of lateral appendages.

(× 237)

Fig. 3. A phase contrast microphotograph of a male inflorescence stained for peroxidase.

(× 90)

Fig. 4. Another phase contrast microphotograph of a male cone stained for peroxidase.

(× 129)

Fig. 5. A vegetative apex of a female plant stained for acid phosphatase localisation. Note the accumulation of the enzyme in the corpus initials.

(× 320)

Fig. 6. A bract producing apex of a female plant stained for acid phosphatase. Note the accumulation of the enzyme in the corpus initials, flank meristem and surface layer of cells.

(× 260)

Note: These photographs are taken from hand sections just to show the localisation of enzymes.

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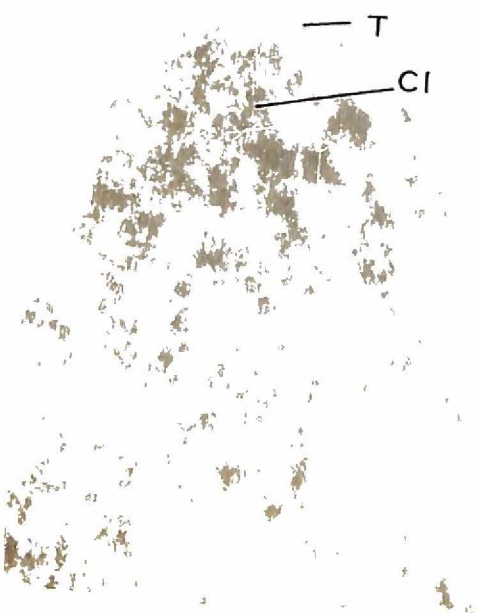

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PLATE XXVIII