A study of the developmental morphology and anatomy of the floral parts of *Triticum aestivum* L.

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ABSTRACT.

The arrangement of outer three layers (i.e. dermatogen, hypodermis, and subhypodermis) in the spikelet apex and floret primordium is similar to that of the vegetative apex. In their origins the lemma, palea, lodicules, carpel and integuments are leaf-like, whereas the floret and stamen primordia are similar to axillary buds.

The integuments arise almost entirely from the repeated divisions of dermatogen cells.

Each stigmatic hair develops by the elongation and characteristic division of a single epidermal cell of the stigma.

A main vascular strand enters the base of each lodicule and by repeated divisions forms a ramification throughout the lodicule.

The procambium appears as an isolated patch of tissue in the position of the future median and lateral strands of the carpel and of the single strand of the stamen. The first appearance of the procambium is restricted to the middle region of the future longitudinal course of the strand. From this point of origin each procambial strand continues its initiation both acropetally and basipetally. A similar pattern of origin and differentiation has been observed for the initiation and propagation of the first protophloem element. It holds also for the first element of protoxylem

in the stamen.

In the funicular strand the differentiation of the procambium and the vascular elements is acropetal.

Comparable stages in the initiation of the carpel and the differentiation of the procambium have also been observed in Avena, Oryza and Secale. The significance of the above observations is discussed.

Some observations are also made on the ultra structure of differentiating protophloem elements of the stamen.

CONTENTS.

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INTRODUCTION.

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There is a considerable literature available dealing with the developmental morphology and histology of grasses, and probably an even more extensive one on the morphology and anatomy of mature spikelets. There are however fewer investigations which are concerned with detailed studies on the actual organogenesis of a grass floret. The most thorough ones are those of Barnard, (1954, 1957a, 1957b), but even these do not resolve the nature of the carpel and lodicules, nor do they contain any information on the initiation and direction of differentiation of procambial (provascular) strands. The origin and the direction of development of the protophloem and protoxylem in the primordia of floral organs does not seem to have been considered by any author so far.

The aim of the present study is to make a thorough investigation of the mode of initiation and early development of the floral organs of Triticum vulgare L. (Bread Wheat). Furthermore, to determine as far as possible the position of origin and direction of differentiation of the procambium, the first element of protophloem in the stamens and carpel, and the same sequence of development in the differentiation of the first element of protoxylem in the stamens, followed by a few comparative observations on some of the commoner cereals. In this way it is hoped to reassess the work which has been done previously and to extend our knowledge beyond what is already available.

REVIEW OF LITERATURE.

The idea of homology of plant parts, especially of leaf and flower, had been recognised by Linnaeus and by Casper Friedrich Wolff in 1759.

Goethe not only recognised these affinities but attempted to explain them in a theory of metamorphosis published in 1790. This was fully developed and supported by de Candolle (1827, 1841). Later this theory has been interpreted figuratively by Gray, Arber and others (James and Clapham, 1935). Gray (1845) stated that the term metamorphosis, as applied to the floral organs is figurative: that is, foliage and floral leaves do not develop from one another, although they may have the same underlying nature. (Cited by Brook (1940).)

Although classical theory has had many supporters, its validity has been challenged from time to time on different bases. McLean Thompson (1934) refuted the classical theory and postulated that the flower is a potentially sporogenous axis, sterile in its basal portion, potentially microsporogenous in the intermediate region and megasporogenous in its terminal portion. The lower emergences of the microsporangia are sterilized and appear, as petals and staminodia.

Foot note:- Some of the references were not available, especially in the cases of earlier ones, but they were included, because although their informations or ideas have had to be gained from later authors, it is felt that it would be desirable to indicate the original sources.

Thomas (1934) also opposed the classical concept of flower interpretation, on the basis of paleobotanical evidences. He held that the angiospermic flower is not the homologue of the vegetative bud. From studies on the reproductive bodies of the Caytoniales, he concluded that the originally fertile organs have undergone sterilization; according to him this transition is much more likely to occur in almost all the higher plants. He further believed that stamens and carpel have derived from fertile branches and leaves from sterile branches. He thought that the carpel of a flowering plant is composed of two fused cupules rather than of a single foliar structure, (cited by Brook (1940).)

Though the importance of the study of internal structures of apical meristems of vascular plants has long been recognised, only in the last three decades has the subject of floral histogenesis attracted much attention. Most of the anatomical work carried out has been concerned with vegetative apices. Relatively few workers made a comparison of vegetative and reproductive apices and even less information is available on the histogenesis of floral organs.

Grégoire (1931, 1938) was the first to attack classical theory on the basis of comparative histogenic investigation of vegetative and floral apices. According to him the vegetative and floral apices are two "irreducible" entities, for the following reasons. (a) The vegetative apex consists of tunica and corpus, whilst the floral apex consists

of a meristematic mantle and a parenchymatous core. (b) Floral appendages are more superficial than vegetative appendages in their origin; no foliar buttresses are found in the flower. (c) Development of procambium in the floral parts is strictly acropetal, while in the vegetative shoot, procambium develops in both directions from the bases of the appendages. (d) The floral apex arises as a new structure from the vegetative apex and not by a transformation of it. He concluded that there is nothing in common between a vegetative shoot and a flower after the formation of the calyx, and in particular, the carpel is an organ without homology to any part of the vegetative shoot. (Cited by Tepfer (1953))

The concept of tunica and corpus theory seems to be more prevalent in the literature concerned than any other yet postulated. This theory was advanced by Schmidt (1924). According to him an angiospermic apex consists of two zones, a surface tunica and an inner corpus. The tunica may consist of one or more layers of cells, where the divisions are ordinarily anticlinal except at the initiation of a leaf. The corpus consists of cells which divide in various planes.

Sharman (1945), to avoid the morphological overtones which were becoming associated with the terms "tunica" and "corpus", used "dermatogen", "hypodermis" and "sub-hypodermis" for the three outer layers of cells, and though here the term "dermatogen" does not carry the same meaning as that of Hanstein (1868).

The application of Sharman's terms "dermatogen", "hypodermis" and "subhypodermis" in a purely descriptive geographic sense is found to be convenient in describing the histogenesis of appendages of grasses by many workers (Bonnett (1953, 1961), Barnard (1954, 1956, 1957).)

Since the work of Grégoire reawakened interest in the meristems of the flowering apex, considerable work has been done bearing on this problem, giving evidence for and against his concept.

Brooks (1940) supported Grégoire's contentions on the basis of his detailed study of comparative histogenesis of vegetative and floral apices in Amygdalus communis, L. He states "there are distinct differences in the initiation and early growth of foliage leaf and floral organs, particularly with reference to the carpel". He holds that the primordia of vegetative leaves are derived entirely from the cells of the tunica, and that the corpus does not enter into their formation in the early stages, whereas the floral organs have deep origin (i.e. all their tissues, except the epidermis, are derived from the corpus). According to him the tunica is four layered in the leaf bud and two layered in the flower bud, becoming one layered at the time of floral organ formation.

The classical concept of carpel and stamen morphology has also been rejected by Satina and Blakeslee (1941, 1943) on similar grounds. They stated, "the initiation and the

development of the leaf, sepal and petal are similar and depend primarily on the activity of the second germ layer (L-II). Whereas the initiation and early development of the stamen and carpel depend primarily on the innermost germ layer (i.e. L-III)." On the basis of their different seats of origin they regarded both stamen and carpel as reduced structures of axial rather than foliar origin.

Wilson (1942), a contemporary of Satina and Blakeslee arrived at a similar conclusion about the nature of the stamen. However, his support is based on comparative studies of vascularization of the stamen and the facts revealed by paleobotany. Wilson postulated his Telome Theory in 1942, according to which the stamen has derived by reduction from a fertile dichotomously branched system or even from a portion of such a system. However this theory has been severely criticised by Canright (1952). He commented, "Wilson's contention (1942) that the three-trace stamens of many ranalean form has evolved from a reduction of a dichotomously-branched system is not borne out by the anatomical evidence at hand. In stamens of Magnoliaceae which bear three traces, normally the two lateral veins are each attached to a different set of cortical bundles, whereas the median vein is attached to a more deep seated stelar bundle. Clearly, in this situation, the possibility of these three staminal veins arising from the dichotomies of a single strong bundle (termed the "stamen fascicle trace" by Wilson)

is very remote.

In addition, ..., these broad ranalean microsporophylls do not have terminal sporangia at veins endings; instead, the sporangia are elongated, often non-marginal, and unvascularized".

Hunt's (1937) support to the axial nature of the carpel is based on the studies of anatomy of carpels of various families. He concluded by saying, "The carpel is therefore considered to have been derived from portions of a primitive dichotomous branch system through the intermediate stage of an unspecialized palmately three lobed appendage"(P.295).

Parallel with these studies on paleobotany (Wilson, 1942) and floral vascular system (Hunt, 1937) an extensive literature has developed dealing with the comparative study of the histogenesis of the foliar and flower buds, that favours the classical interpretation of the floral appendages.

Philipson (1946) believes that the reproductive apex is not a new structure but is formed by the transformation of the vegetative apex. According to him, the differences in the zonation of the vegetative and reproductive apices appear to be a result of different directions of growth rather than of such fundamental nature as Grégoire attributed to them. Popham and Chan (1952) arrived at the same conclusion, after studying in detail, the gradual transition of the vegetative apices into reproductive of Chrysanthemum. These observations are also in agreement with that of Vaughan (1953).

Newman (1936) surveying the floral primordia of *Acacia longifolia* and *A. suaveolens*, favours the classical interpretation of the floral organs. Specifically, he concluded that the carpel is a single, folded, foliar structure and is lateral in its position on the floral apex.

Reeve (1943) investigated the comparative ontogeny of the inflorescences and the axillary vegetative shoots in *Garrya elliptica*. He holds that there are no histological differences between an axillary floret primordium (male or female) before the emergence of its prophylls and the axillary foliar bud, in comparable stages of development, except for the narrower medullation zone (rib meristem) in floret primordia.

According to Philipson (1947) and Vaughan (1953) the floret (or flower) primordia are equivalent structures to the axillary buds.

Inspite of several recent investigations, there are many problems connected with the origin and early development of axillary buds that still remain unsolved. One of them is the origin of the meristematic cells from which the buds arise. Philipson (1948) has described the origin of flowering branch buds from the meristem "detached" from the apical meristem. On the other hand, Majumdar and Datta (1946) describe the origin of bud meristems from vacuolating tissue in the axils of the leaves.

Engard (1944) has made an extensive study of organogenesis in four species of *Rubus*. According to him the

foliage leaves and all floral appendages are initiated by the enlargement and periclinal division of the inner tunica layer, along with periclinal division of a few cells of the outer layer of the corpus. His observations are in conformation with those of McCoy (1940) on Frasera carolinensis and Boke (1947, 1949) on Vinca rosea.

The literature concerning the floral histogenesis, especially the carpel morphology has been competently reviewed by Swamy (1945) and Joshi (1946). Swamy supported the classical concept of carpel morphology and refuted the others, especially the "polymorphism" theory of Saunder (1925) on the following grounds (1) no anatomical evidence, (2) very complicated and (3) does not explain all angiospermic flowers. Joshi concluded that a considerable difference may exist in most of plants between the organogenesis of vegetative and floral buds, but these differences are not "irreducible type" as maintained by Grégoire. Furthermore, the fact that the differences do occur does not invalidate the conclusion of homology when ontogeny is similar.

Recently Tepfer (1953) examined and compared histogenesis of the vegetative and floral apices and their appendages in Aquilegia and Ranunculus. He found that the foliage leaves and all floral organs arose in the same manner and therefore, he concluded the leaf-like origin of floral appendages. However, he distinguished the mode of development of foliage leaves, bracts, sepals and carpels

from that of petals, stamens and staminodia in Aquilegia (P.538).

Picklum (1954) investigating the developmental morphology of the inflorescence and flower of Trifolium pratense L, observed that the foliage leaves and floret primordia are similar in their origin. Each originates by the activation of cells of the inner layer of the tunica and one or two adjacent layers of the corpus. However the initiation of the floral organs depends on the activity of the inner layer of the tunica, he stated, "the cells of the second tunica layer enlarge anticlinally and some periclinal divisions occur prior to the initiation of floral appendages".

Though an extensive literature is available on the developmental morphology of grasses, including most of the grasses of economic importance, the number of publications on Triticum is still comparatively small.

The gross developmental morphology of the shoot and reproductive apices of grasses received considerable attention at the end of the nineteenth century as shown by the works of Trécul, Goebel (1884) and Payer (1857).

Since the work of Trécul and Goebel, an increasing number of investigators have made observations on the origin and development of the inflorescences and the floral parts of a number of Graminaeae species.

Among the excellent publications dealing with the morphology of grasses those of Arber (summarized in her

"Gramineae" 1934) are the most extensive; she is mainly concerned with the morphology of adult inflorescences and flowers of cereals, bamboos and grasses.

Percival (1920) in his study of the wheat plant shows that stamens arise as rounded papillae, and the carpel as a crescent shaped ridge closely encircling the floral apex. He thought that the ovule derives from the morphological apex of the floral axis. According to him the order of differentiation of floral appendages is glumes, stamens, carpel, and then the palea and lodicules simultaneously.

Morphology of the shoots of grasses during the development of inflorescences and flowers have been investigated by many workers.

Evans and Grover (1940) studying the developmental morphology of the growing points of the shoot and the inflorescence in grasses observed, "the secondary protuberances of the inflorescence have the same relative position on the primary axis, as the vegetative buds and are their homologues".

The contribution of Bonnett (1935, 1936, 1937, 1940, 1953, 1961) to morphology and histology of inflorescences and floral appendages of grasses, chiefly cereals, is outstanding. With regard to the formation of various floral organs in Zea (1953) and Avena (1961), Bonnett recorded that both the floret and stamen primordia are equivalent to the axillary vegetative buds and furthermore, that the lodicules are axial in nature. He employed Sharman's terminology for

describing the arrangement of the outer three meristematic layers in the floral apices, as well as for the histogenesis of the floral appendages.

The organization of cells within shoot and floral apices, and floral histogenesis of other monocotyledons have also been studied.

Sass (1944) holds that the initiation and development of the perianth and stamens, in the tulip, are similar to those of vegetative leaves.

Stant (1952) thought, while working on the shoot apices of some monocotyledons, that the growing point (meristem) is organized into four distinct regions, the cells of which differ in appearance, properties and in their direction of division. Rather surprisingly she did not observe any periclinal division in the outer layer of the tunica in relation to leaf origin.

Sharman distinguished the outer three layers of the apex as dermatogen, hypodermis and subhypodermis and employed these terms in a purely geographical sense (as mentioned earlier). He regarded floret and stamen primordia as bud-like in their origin and the gynoecium as monocarpellary in Anthoxanthum (1960).

Esau (1953) included cereals in her publication on plant anatomy and concluded that the grass gynoecium arises as a single unit, and does not reveal ontogenetically the three carpellate structure, as associated with the Graminae flower.

Holt (1954) investigating the initiation and development of inflorescences of Phalaris arundinacea L. and Dactylis glomerata L. reported that the outer layer of the corpus also contributes to the development of foliage leaves, lemma and pistil, in addition to the two tunica layers, while glumes, palea and lodicules are derived exclusively from the tunica. He also holds that the carpel develops as a single foliar structure and the ovule is produced from the residual dome of the apical meristem.

On the contrary, Chandra (1963), after detailed study of the spikelets of various species, regards the gramineous gynoecium as tricarpellary in nature and the solitary ovule being borne at the fused margins of the two lateral carpels. However, all his observations seem to be confined to adult material.

Surkov (1961) studied morphogenesis in Graminae (1953-1958) and concluded "That the Graminae floret is a branching shoot, the lateral parts of which, as well as central growing point, having passed a certain course of development have acquired the capacity of the accomplishment of the function of sexual reproduction".

Until recently, no particular attention was given to the organogenesis of the Triticum floret, nor to that of any other member of the Graminae, although the histology of young shoot apices has been studied by Rösler (1930) in Triticum.

Barnard (1954, 1957a, 1957b) investigated the histogenesis of the inflorescence and the floral parts of various members of two monocotyledon families (Gramineae and Cyperaceae). His contentions are very similar to that of Sharman's (1960), except that he regards the gramineous gynoecium as composed of four or at least three carpels. His work on histogenesis of the inflorescence and flower of Triticum aestivum makes the basis for the present study.

Origin and differentiation of procambium and first vascular elements.

There is a divergence of opinion about the origin of procambial cells. Philipson (1947), in a discussion of the origin of the procambium of the stem, stated that the maturation of the cells forming the pith and cortex leaves a zone of narrow cells between, which remains meristematic, and this is the zone that constitutes the procambium. He extended the same definition for the initiating cells of the procambium in the leaves and reproductive apices. Tepfer (1953) holds that the initiation of the procambial cells is the result of longitudinal divisions of certian cells and comments that it is a criterion more nearly capable of objective application.

Another factor that confuses the study of procambium is the lack of agreement on the characteristics, that can be associated with the precursor of the procambial cells. How-

ever Tepfer (1953) and Esau (1954) postulate that the youngest procambial cells can be identified, by their position with respect to the vascular tissue in the mature organ, darker staining reaction, elongation and the plane of divisions, in these cells.

Grégoire held that in the inflorescence, peduncle and the floral receptacle, the procambial strands develop in an acropetal manner quite independently of the appendages. The traces do not originate in any lateral organ, bracteal or floral, they extend towards these organs, and the vascular system of the floral axis is not made up of descending traces.

In her excellent review on origin and development of primary vascular tissues in seed plants, Esau (1943) commented, "Interestingly enough, Grégoire appears to be the first to describe acropetal differentiation of floral traces as a general phenomenon. A perusal of literature on the development of flowers shows that workers, if they mention the subject at all, usually report basipetal differentiation of floral bundles. According to Grélot (1898) the procambium of floral bracts of several species differentiated first at the base or in the free portion of the organs, then proceeded acropetally and basipetally". Lanessan reported basipetal differentiation of procambium and xylem in all floral organs of Primula. Instances where both types of differentiation occur in the same flower have

also been recorded, Lanessan (1877) reported basipetal differentiation of procambium in some organs and acropetal in others in Rivina. (Cited by Esau, (1943)).

According to Brooks (1940), the median procambial strand in the developing carpel of Amygdalus, first differentiates in the central region of the primordium, on the abxial side. It then develops acropetally and basipetally until it connects up with the vascular system of the torus lower down.

Lawalrée (1948) referring to certain Compositae, states that the procambia of the corolla, the stamens and carpels arise in isolated loci near these structures and then differentiate in two directions. Arnal (1945) reported a similar course of procambial differentiation for floral parts of certain Violaceae.

Acropetal differentiation of the procambium in floral parts of dicotyledons is reported by Miller and Wetmore (1948) in Phlox drumondii Hook, by Tepfer (1953) in Aquilegia and Ranunculus, by Engard in Rubus, by Philipson (1946) in Bellis, Popham & Chan (1952) in Chrysanthemum and by Satina and Blakeslee in Datura. Boke (1940, 1949) working on Vinca rosea L. found, in sepals and petals, the course of differentiation of procambial strands convincingly acropetal, but for stamens and carpels he states "In stamens and especially carpels, procambial strands are not obvious until the associated primordia are already conspicuous protuberances. Although no convincing evidence of procambial discontinuity

was found in stamen and carpel primordia, the direction of differentiation is difficult to demonstrate. All procambial cells in a stamen or carpel trace appear to be in about the same stage of development at a given time. An acropetal "wave" of differentiation is not evident", (P.541).

The origin and direction of differentiation of the procambium in the monocotyledons (especially of grasses) has received comparatively little attention. The investigators concerned do not seem to mention the subject at all for floral appendages and even for leaves, very limited information is available on the origin and differentiation of the procambium.

Basipetal differentiation of procambial strands has been reported by Guillaud in the leaves of some members of the monocotyledons and by Mollendore (1948) for leaf traces in Brachypodium. Hsü (1944) observed bipolar differentiation of procambial cells in the traces of cataphylls as well as in the vegetative leaves of Sinocalamus, and Bugnon (1921, 1924) in the leaves of Dactylis and Melica.

The leaf development of Zea mays has been investigated in detail by Sharman (1942). According to him the median bundle differentiates acropetally within the leaf, (but he also assumed that the lower part of the median procambial strand may differentiate basipetally in the stem region), whereas the differentiation of the large lateral bundles is bipolar and the smaller bundles (bundles inter-polating

between the larger laterals) differentiate basipetally and appear first near the apex of the leaf after the larger laterals reach their highest positions.

Support for basipetal differentiation of procambium in some dicotyledons is also given by surgical experiments. Clowes (1960) while discussing the differentiation and origin of procambium stated, "There is nothing inherently improbable about basipetal differentiation of the procambium and it is known to occur after certain surgical treatments. Perhaps the clearest example comes from the work of Camus (1949) who has shown that buds, grafted on the fleshy roots, induce the formation of procambium in the parenchyma of the root. Similar results have been obtained by applying synthetic auxins to spots on the root. We may imagine a bud producing a hormone which flows down the root tissue and we should expect basipetal differentiation of the procambium to follow the path of the hormone. Similarly, in Ball's experiments in isolating apices of Lupinus from the procambium of the stem by incision, it is evident that the newly formed procambial cylinder in the apex becomes connected to the older procambium by basipetal differentiation within the parenchyma separating the two procambial systems (Ball, 1952)."

The importance of the study concerning the origin and direction of differentiation of vascular elements (both phloem and xylem) in floral appendages, though long been recognised, has only recently attracted any attention. For

this reason very scanty information is available on this aspect and even this is limited to dicotyledonous flowers.

The independent origin and bipolar differentiation for xylem elements has been reported by most workers. In some cases xylem elements originate at two points in the same bundle of an organ, and then the upper differentiates basipetally and the lower acropetally until the two meet.

Cheadle and Whitford (1941) concluded, after surveying the vast number of monocotyledoneae, that their metaphloem contain no sieve cells in the strict sense, but sieve tubes are invariably a part of the phloem in all organs of the plants examined.

The origin and direction of differentiation of the first protophloem element is still controversial. In the literature available, in most cases it is reported as differentiating acropetally. However in a few instances the basipetal course of differentiation of the phloem has also been observed, (Guillaud (1878), Hasselberg (1937), Lignier (1890) and Priestley and Swingle (1929).) (Cited by Esau (1943).)

Barnard (1957) examined the vascular structure of the Triticum carpel but unfortunately used material too old to show the initial stages of differentiation of vascular elements.

Nomenclature

Floret

The term floret has been used following Hitchcock (1935). He has included under this term, lemma, palea, lodicules, androecium and gynoecium.

Style

The receptive part of the female reproductive organ in higher plants is regarded as the stigma, which is usually provided with a papillate surface. In a mature wheat floret, the whole length of each "style" is covered by long filamenteous hairs (stigmatic hairs) and presumably receptive throughout the length. For this reason the term stigma rather than style has been used for the whole length of the structure.

Periclinal divisions

A cell is said to divide periclinally when the new wall between two daughter cells is parallel (or approximately so) to the outer surface of the organ.

Anticlinal divisions

A cell is said to divide anticlinally when the new wall between the two daughter cells is perpendicular (or approximately so) to the outer surface of the organ.

Use of ' and ",' has been used to indicate the earliest stages in the initiation of organs, for example, where the lemma is represented only by one or two dividing hypodermal and dermatogen cells, it is labelled as L', whilst the

developing or mature lemma is labelled as L. FP" is used to distinguish the antero-posterior flattened stage of the floret primordium (from other stages) that occurs just before the appearance of the lateral stamens.

Carpel

In the light of the observations which have been made, the gynoecium is regarded as being monocarpellary in nature. Thus, the term carpel is used instead of gynoecium throughout the account, and in the illustrations.

Plane of glumes

A longisection passing through the mid-regions of the floret primordium (or floral axis), anterior stamen, lemma and glume is regarded as the section in the plane of glumes.

Materials and Methods.

Triticum aestivum - Capelle Desprez (a winter wheat) and Svenno (a spring wheat) were used for this study. It was decided that it would be an advantage to use known cultivars wherever possible, because this would provide uniform material, whose behaviour should be similar in successive years.

The winter wheat Capelle Desprez has to be sown before the end of February, to ensure that it would flower during the coming summer.

Svenno, being a spring wheat will produce flowers when sown at any time during the spring or even in summer as late as July. It is thus more useful than Capelle, because a number of successive sowings will provide a continuous supply of suitable material.

Grains were sown in wooden boxes (30 x 37½ x 15 cms) of garden soil, to each box about 28 gms of fertilizer was added.

After sowing, the boxes were kept in the unheated greenhouse for some time, and then later in the spring were transferred to the open, under a bird proof net.

Plants grown under the conditions described above, usually have young inflorescences six weeks after sowing. At this early stage the inflorescence is very short, with spikelet primordia developing acropetally.

After their initiation, the florets reach maturity in about four to six weeks.

The flowering in Triticum occurs only under the conditions of long days (i.e. about 14 to 16 hours day length).

Dissections were made at intervals, from the time the plants had the third green leaf with its lamina exposed as far as the ligule.

From inflorescence initiation onwards apices were dissected out, in a moist atmosphere, at intervals of four days. The appropriate material was selected and trimmed. In the case of young inflorescenses, the last exposed vegetative leaf was not removed. Likewise glumes in the case of spikelets and lemmas, in the case of florets, were not dissected out. These structures not only help in the orientation of the material later but also protect it from direct exposure, and rough handling during dehydration and embedding.

The material was fixed in one of the following:-

(1) Formalin and citric acid.

(2) Formaldehyde and glacial acetic acid (F.A.A.)

(3) Chromic Acid (Nevaschin's fluid modified).

(4) Gluteraldehyde (4%) in phosphate buffer.

Most of the material was fixed in F.A.A; although it causes a considerable disorganisation of cytoplasm, it penetrates rapidly and enables the cell wall to be stained easily with tannic acid. This then contrasts well with the slightly shrunken cell contents. In addition this very effect also proves useful, in distinguishing the young procambium cells from their neighbours. In the early stages of

procambial strand initiation, differences in the average density of the disorganised cytoplasm and its staining, make this possible.

The material was fixed in F.A.A. for at least 24 hours, washed in 70% alcohol, dehydrated in a conventional way, by using a graded ethyl alcohol and chloroform series. Then fine paraffin chips were added for dissolution in chloroform containing the material (which was also gradually heated up in the oven or on a hot plate) up to saturation point, for one to two hours. Then the material was passed into molten paraffin and left overnight in an oven at 60°C. After passing through several changes of molten paraffin, to remove all traces of chloroform, it was embedded in paraffin.

Although various types of waxes with varying melting points were tried, for most of the work "Fibro wax" and "Paraplast wax" was used and melting points 56 - 58°C were found most satisfactory. The best blocks were obtained by cooling the wax in iced water.

Blocks were cut on a "Unicam" racking microtome and 5 - 10 micronsthick sections were obtained.

Both transverse and longisections were utilized in this investigation, but on the whole transections were found to be more useful in detecting the initial stages of procambium and that of first vascular elements.

Although only tried relatively late in this study, gluteraldehyde was found very satisfactory, as it fixes the

cellular contents better and cell walls of material fixed in this solution stain readily.

The material was fixed in gluteraldehyde from 4 - 12 hours (overnight). After washing with phosphate buffer (three changes of 10 minutes each), material was either taken up in an alcohol and propylene oxide series and embedded in paraffin, or transferred to 70% alcohol or F.A.A. for storage.

For orientation of the small objects (such as young spikelets and florets) the method of using sodium alginate (Sharman 1960) was adopted.

Staining

Sharman's staining method (1943) was employed as it stains immature cell walls darker, so that they can be picked up even when they are very young, this being the sole interest of the study. (However, it was found necessary to use a slightly stronger solution of Safranin).

Other staining methods were also given trial, for example (i) Methyl green and pyromin y (extracted in chloroform), (ii) Safranin and fast green and (iii) blue recorcinol, used for detecting the presence of callose in young sieve elements. These, however, gave no advantage over the standard tannic acid iron alum method.

Photography

Floral apices and dissected florets were photographed under a binocular microscope, using a camera (Exa I) attached to one of the oculars, following Sharman's (1947) technique

for photographing shoot apices in the Gramineae.

Anatomical slides were photographed either under an ordinary compound microscope or with a Zeiss "photo-microscope".

Both Kodak (panchromatic) and Ilford (Fp3) films were used for black and white photographs and Ilford (colour series) for coloured photographs. Most of the drawings were made with the help of the microprojector, but a few were obtained by inking and bleaching the photographs in diluted iodine solution followed by sodium thiosulphate solution.

Bleaching and Clearing Method.

The methods described by Bonnett (1961), Bisalputra and Esau (1964) were attempted, although in preliminary trials xylem elements could be seen clearly, details of the phloem elements were quite indistinguishable, so these methods were soon abandoned.

OBSERVATIONS.

(1) Morphogenesis.

(a) Glumes and Lemmas.

The glumes and successive lemmas arise as alternate lateral ridges, each of which more than half encircles the spikelet axis, Plate I A-C at G and L respectively. In the material studied usually two empty glumes and five to seven lemmas with their axillary florets develop acropetally.

For some time the growth is uniform all round the margin of the young lemma, but soon after the central region can be distinguished by its rapid growth from the rest of the blade, indicating the commencement of the development of an awn, see Plate 2 A-C at AW.

(b) Floret Primordium.

The floret primordium is seen as a small rounded papilla in the axil of the youngest (last formed) lemma as shown at Fp in Plate IA and B. It grows out and becomes almost hemispherical in shape.

(c) Stamens.

The hemispherical appearance of the floret primordium is transitional, and soon it is possible to see that the floret apex is becoming wider laterally, as may be noted at Fp" in Plate 2A and B, and Plate 4 at b. This lateral expansion of the floret primordium foreshadows the position of the future two lateral stamens, as indicated by a slightly later stage in Plate 2A at Ls. A still more advanced stage is depicted in Plate 2B at Ls, where the primordia of two

lateral stamens have assumed the shape of rounded papillae and are visible on either side of the floret apex.

The third or anterior stamen develops after the two lateral ones, see Plate 2B and C at As, where it may be noted that the anterior stamen is smaller than the two laterals.

Although the two lateral stamens can be detected before the anterior one, the latter apparently grows faster than the former because it not only catches them up in size, but in some cases even gets ahead, thus reaching the dimpled stage (i.e. four rows of microsporogenous tissues have started differentiating inside it) before the lateral stamens.

Though the lateral stamens are the first to be seen morphologically, this does not necessarily mean that they are initiated before the anterior one. Their initiation may be simultaneous, (see Histogenesis).

The primordia of all three stamens soon become elongated and develop quadrilocular anthers. From this stage onward the growth of the stamens is relatively very slow; they increase in size but show no striking morphological changes. The filament is more or less invisible in the earlier stages of development of the stamen, but gets very much elongated at the approach of maturity.

(d) Palea.

The palea arises as a narrow ridge of tissues on the adaxial (or posterior) side of the floret axis see

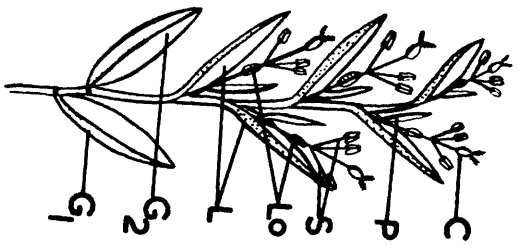
Figure I.

Fig. 1.1. indicating the arrangement of two empty glumes and acropetally developing lemmas (each with its axillary florets) on the spikelet axis.

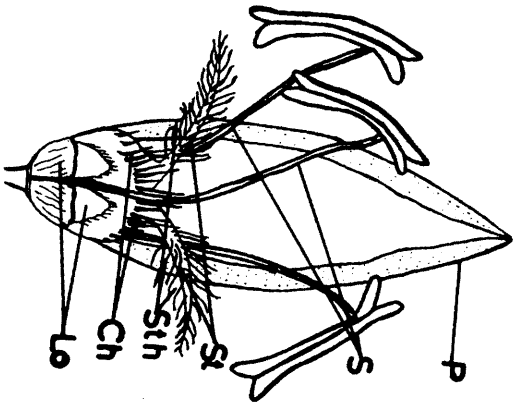
Fig. 1.2. A view of the Triticum floret from the anterior side (the lemma has been removed).

Fig. 1.3. Diagrammatic representation of the transection of a floret.

Fig. 1.4. An anterior view of the carpel, a portion of the ovary wall has been dissected out to show the position of the ovule and its micropyle.



1.1



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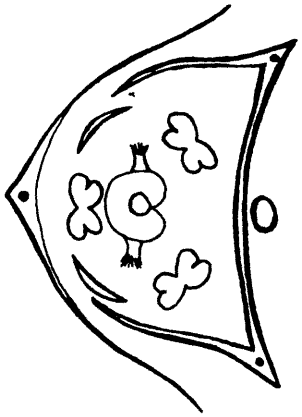

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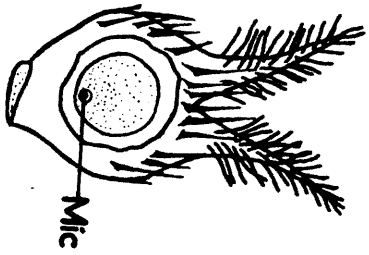


1.4

Figl

Plate IC at P. The appearance of the palea most probably synchronises with that of the stamens. Even in the early stages of development the bi-keeled appearance of the palea is evident, as it grows more rapidly on its lateral edges than in the centre. The projecting lateral edges of the palea from the posterior side of the young florets may be noted in Plate 2 A, C and D at P. However, the growth of its centre soon overtakes that of the lateral wings.

Before the differentiation of the antherlobes in the stamens, growth of the palea is remarkably slow, so that when the stamens have well developed anthers, the palea is only half their length. However, as soon as the growth of the stamens slows down, the palea grows faster and overtops them.

(e) Lodicules.

A floret has two lodicules, each of them occupying an antero-lateral position on either side of the anterior stamen. In its earlier stages of development, each lodicule appears as a small ridge of tissue between the anterior and lateral stamens. Soon after it assumes a boat shaped form, see Plate 5 E and F.

A mature lodicule is very much swellen at its base and gradually gets thinner towards its tappering tip, see Fig. 1.2 at Lo and Plate 3. The lateral sides of the lodicules, next to the lateral stamens, are provided with wing-like structures, as may be noted in Fig. 2 at Lo. The margin

Figure 2.

A three dimensional view of a Triticum floret, showing the arrangement of floral parts, and also illustrating the positions of vascular strands in stamens and carpel at different levels.

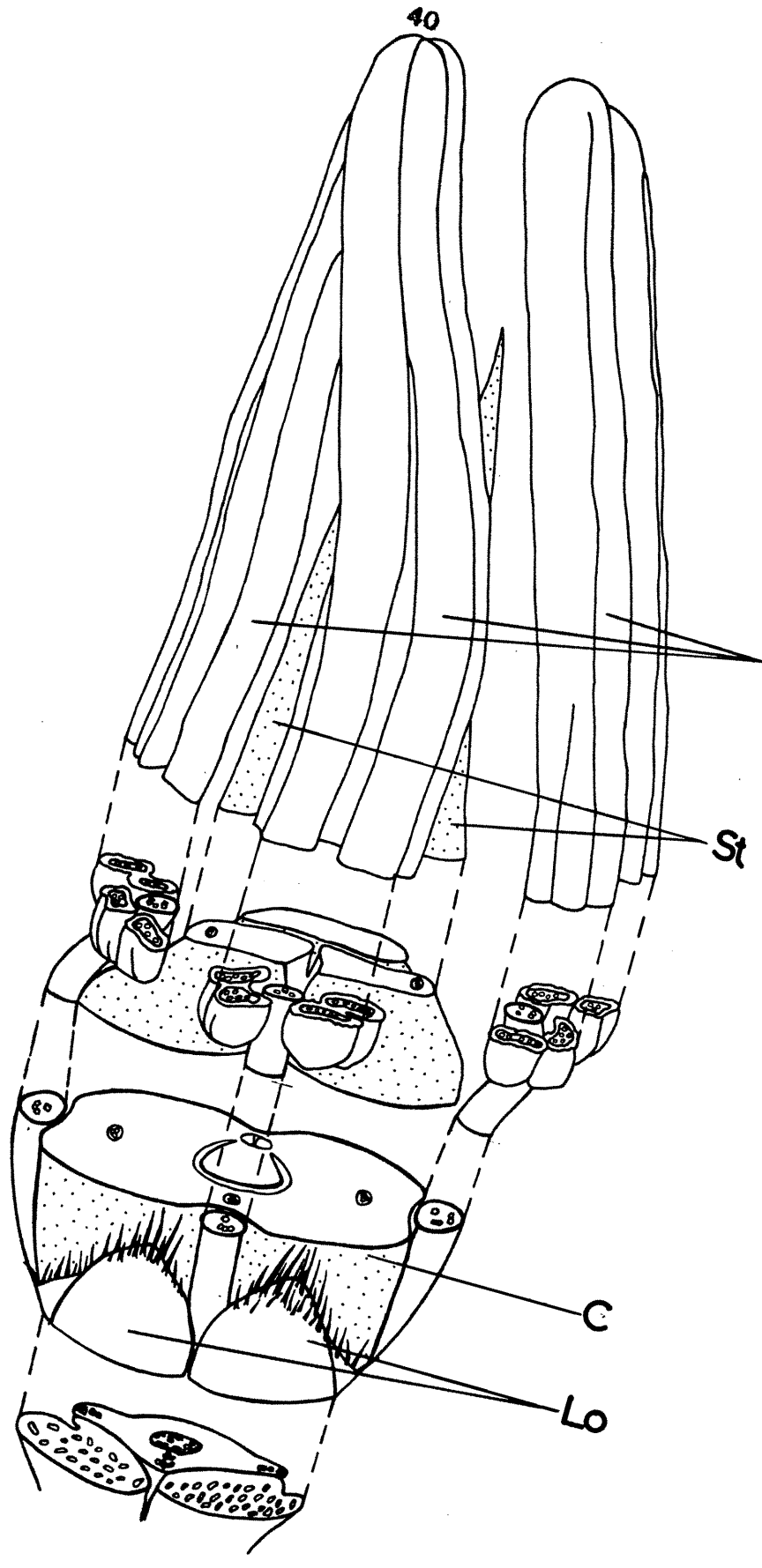


Fig2

of the lodicules are covered with unicellular hairs. Each hair is long and tappering, with the nucleus in its more or less swollen base and the cell wall is not markedly cutinized. These hairs tend to be longer and more numerous on the upper margin than on the sides.

It is rather difficult to detect morphologically the earliest stages in the development of lodicules, therefore, it cannot be ascertained whether stamens and lodicules originate simultaneously or one after the other. However, judging from anatomical observations, (see Histogenesis) it seems likely that the lodicules may appear after the stamens.

(f) Carpel.

The carpel makes its beginning after all three stamens have assumed the shape of distinct papillae. In its early stages of development, it is in the form of a small crescent round the floret apex, with its two edges facing the palea and its back next to the anterior stamen, as may be noted in Plates 4 and 5A at C. At first, the growth of the carpel is most rapid on the anterior side of the floret apex, then it gradually spreads towards the posterior side, with the result that a cowl shaped structure is formed and the apex of the floret primordium becomes completely enclosed in it at its base, as shown in Plate 5B and C.

At first, the growth of the cowl is uniform all round its circumferance, but soon the margins of the cowl opposite to the laterally placed anthers grow much faster

than the other portions, forming primordia of the two stigmas.

The primordia of the two stigmas make their appearance fairly early in the development of the carpel, as may be seen in Plate 5D at St. It should also be noted that the carpel still shows a small opening at the top.

Plate 5C depicts an even earlier stage in the differentiation of the primordia of the two stigmas, where two small papillae (one opposite to each lateral stamen) can be distinguished. It should be noted that the primordia of the stigmas are discernible even before the two edges of the carpel are completely fused on the adaxial (posterior) side of the floret apex.

Later stages in the development of the stigmas can be seen in Plate 5E and F, where each of the two stigmas has assumed the shape of a cone.

Later on, the carpel grows both in height and diameter, the middle portion (or portion slightly above it) of the ovary particularly, continues to increase in diameter and thickness until it is larger than the base, resulting in the carpel becoming obovate. Most of the increase in height is due to the elongation of the two stigmas, see Fig. 1.2. The top of the ovary closes by marginal growth of the carpel, although closure is never complete. A small opening persists as the stylar canal. (see Histogenesis).

The upper half of the ovary becomes covered with a number of epidermal hairs, Plate 5 G and H. Each hair is

long, tappering, monocellular, and uninucleate, with the nucleus in its slightly bulbous base. The cell walls of most of the hairs are thin and slightly cutinised, but some have very thick walls and stain darkly, most likely due to the deposition of cutin.

At maturity, the tips, the inner surfaces and part of the lateral surfaces of both stigmas are nearly covered to the bases with long filamentous stigmatic hairs which form a plumose group. An early stage in the production of these is indicated in Plate 5H at Sth. Most of the exterior surface of the stigmas is without stigmatic hairs. Each stigmatic hair, when mature, consists of four columns of cells. The end walls of the cells of each column do not coincide but occur in a spiral. Each stigmatic hair terminates in a single cell. This is not an odd terminal cell, but one of the terminal series of four cells which is highest in the spiral. The tip of each cell is rounded and protrudes outward and upwards towards the tip of that particular stigmatic hair, overlaping the base of the cell immediately above it. The protruding ends of the cells provide an easy entrance for the pollen tubes.

The cells of the stigmatic hairs are thin-walled, having a nucleus more or less in the centre of the cell.

(2) HISTOGENESIS.

Figure 3 shows a longisection* of a terminal spikelet, cut in the plane of glumes. In this the basal florets are already well developed, while the upper ones are just being initiated, thus depicting the acropetal development of the florets and their substending lemmas.

In the material studied, two empty glumes and usually five to seven lemmas with their axillary florets develop on the spikelet axis in acropetal succession. Of these florets usually not more than three to four develop to maturity, the more distal primordia cease to develop at various stages.

Figures 4.1 and 4.5 indicate that the spikelet apices consist of three layers covering a central core. Following Sharman (1945) these layers will be refered to as dermatogen, hypodermis and subhypodermis respectively and are shown labelled as D, H. and SH. The subhypodermis is often a less clearly distinguished layer than the other two and perhaps could be regarded as the outer region of the core.

(a) Lemma.

The lemmas develop almost exactly in the same way from the axis of the spikelet as leaf primordia do from the vegetative axis.

The first observable indication of the initiation of a lemma is the periclinal division of three to four

* Longisections are in the plane of glumes, unless otherwise stated.

Figure 3.

Longisection of a young terminal spikelet (taken in the plane of glumes), illustrating the acropetal insertion of successive lemmas and their axillary florets, and also various stages in the development of the lemma (L_1-L_5), floret primordium (Fp), anterior stamen (As) palea (P') and the initiation of carpel (C').

Note:- All scales shown in the illustrations are equal to 100 micron.

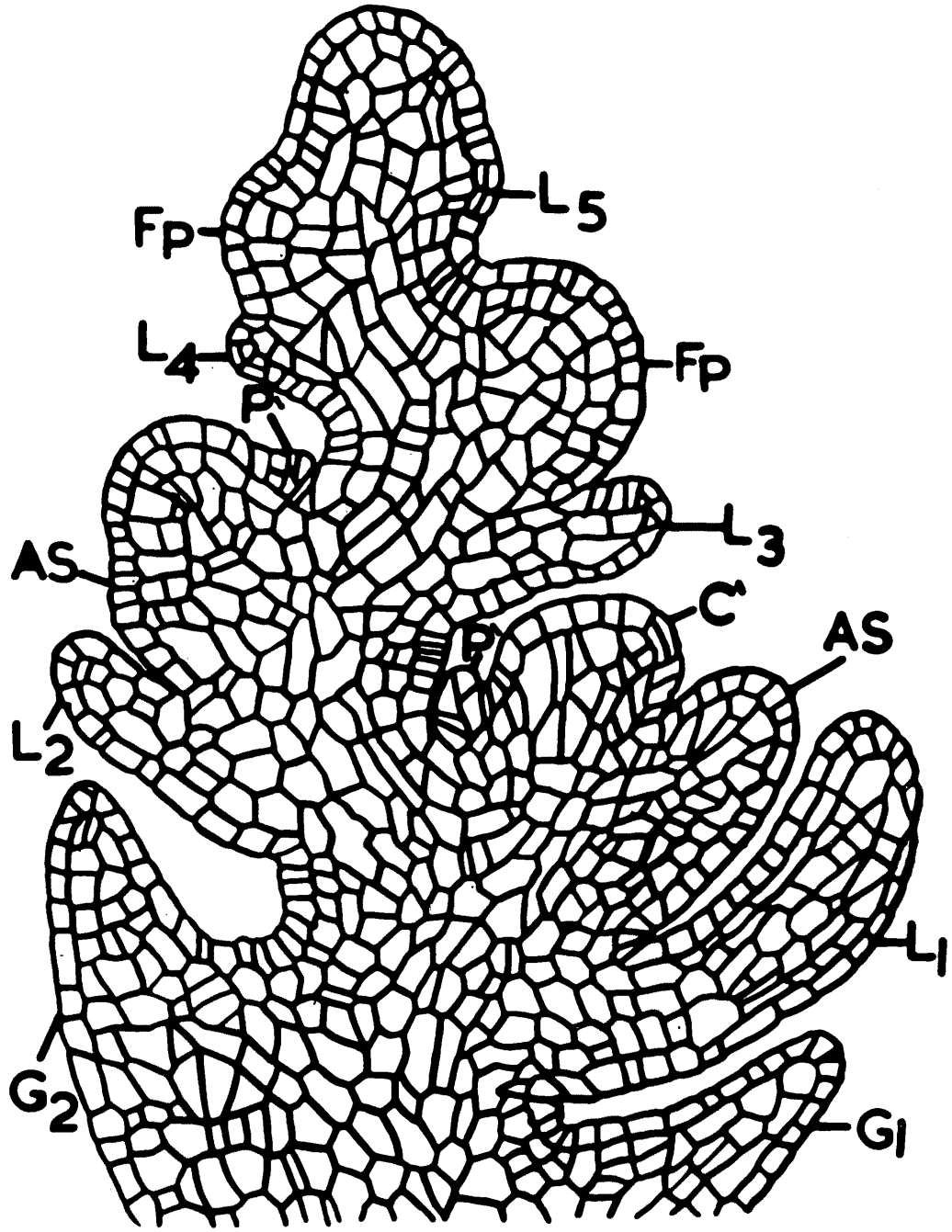


Fig3

hypodermal cells in the vertical plane, which is generally followed or accompanied by that of two adjacent dermatogen cells, as shown in figures 3 at L^5, 4.1 and 4.5, and Plate 7A at L'. These divisions commence from the centre of the region of insertion of the future lemma, as may be seen in figures 4.2 and 4.4, representing the spikelet apices in transection. But soon after, this meristematic activity extends transversely around more than half the circumferance of the axis. Figures 3 at L^4, 4.3 and Plate 7B at L, illustrate slightly later stages, where hypodermal and dermatogen cells have undergone repeated periclinal divisions so as to form a group of cells. It is this buldge of cells which probably constitutes the first sign one sees of the lemma, when examining dissected material.

Subsequent development of a lemma takes place by the periclinal and anticlinal divisions of the dermatogen and hypodermal cells and their derivatives.

It can be seen from Figs. 3 at L^4, 4.3, 4.5, 4.8 and Plate 7B at L that dermatogen cells probably contribute more than hypodermal cells, at least in the early stages, to the development of the lemma. Repeated divisions of the dermatogen cells, in the above illustrations, also indicate that most of the early growth is due to apical meristematic activity.

The cells on the lower side of the lemma primordium divide more actively than the upper ones, with the result

that more cells are produced on the lower surface than the upper. This causes the lemma to turn upwards, see Fig. 3 at L^1 and L^2 and Fig. 4.9.

(b) Floret Primordium.

The floret primordium arises in the axil of a lemma. It is comparable in its mode of initiation to a spikelet primordium or an axillary vegetative bud. Thus it can be regarded as a cauline structure.

The first indication of the initiation of the floret primordium is usually the periclinal division of three subhypodermal cells in the axil of the last formed lemma. These cells undergo more periclinal divisions, with the result that each produces a more or less horizontal tier of two to four cells, so forming a group of cells that pushes the hypodermal and dermatogen layers slightly outwards, as indicated in figures 4.3, 4.5 - 4.7 and Plate 7B at Fp. The periclinal divisions in the hypodermal cells are generally observed on the upper side of the future floret primordium, (Fig. 4.3. Plate 7B at Fp) and may occur sometimes on the lower side as well. A single periclinal division has been observed regularly in one hypodermal cell towards the centre of the floret initial, this cell enlarges conspicuously (presumably before its division though this is not certain) while the other dividing cells do not, as depicted in Figs. 3 at Fp. (in the axil of L^4) 4.3 and Plate 7B at Fp. At no stage do the dermatogen cells divide by other than anticlinal divisions.

Figure 4.

Fig. 4.1. Longisection of a young spikelet apex showing periclinal divisions in hypodermal and dermatogen cells at the initiation of the lemma.

Fig. 4.2. Similar initial stages of lemma seen in transection.

Fig. 4.3. Longisection of a slightly older spikelet indicating the pattern of development of lemma (L) and floret primordium (Fp).

Fig. 4.4. Transection of the same, at the level of the lemma.

Fig. 4.5. Longisection of a young apex from another spikelet showing stages in the development of lemma and floret primordium.

Fig. 4.6. Longisection of a young spikelet, depicting the arrangement of three meristematic layers in a floret primordium, before the development of floral organs.

Fig. 4.7. Longisection of a floret primordium showing initial stages of anterior stamen and palea.

Fig. 4.8. Longisection of another floret primordium, slightly more advanced than 4.7, indicating the early development of anterior stamen and palea.

Fig. 4.9. Longisection of the same floret (drawn for 4.8) but 40 micron off the median, showing where the lodicule is being initiated by periclinal divisions in two hypodermal cells. The activity of dermatogen and hypodermal cells in the region of palea seems to be more here than in the mid plane.

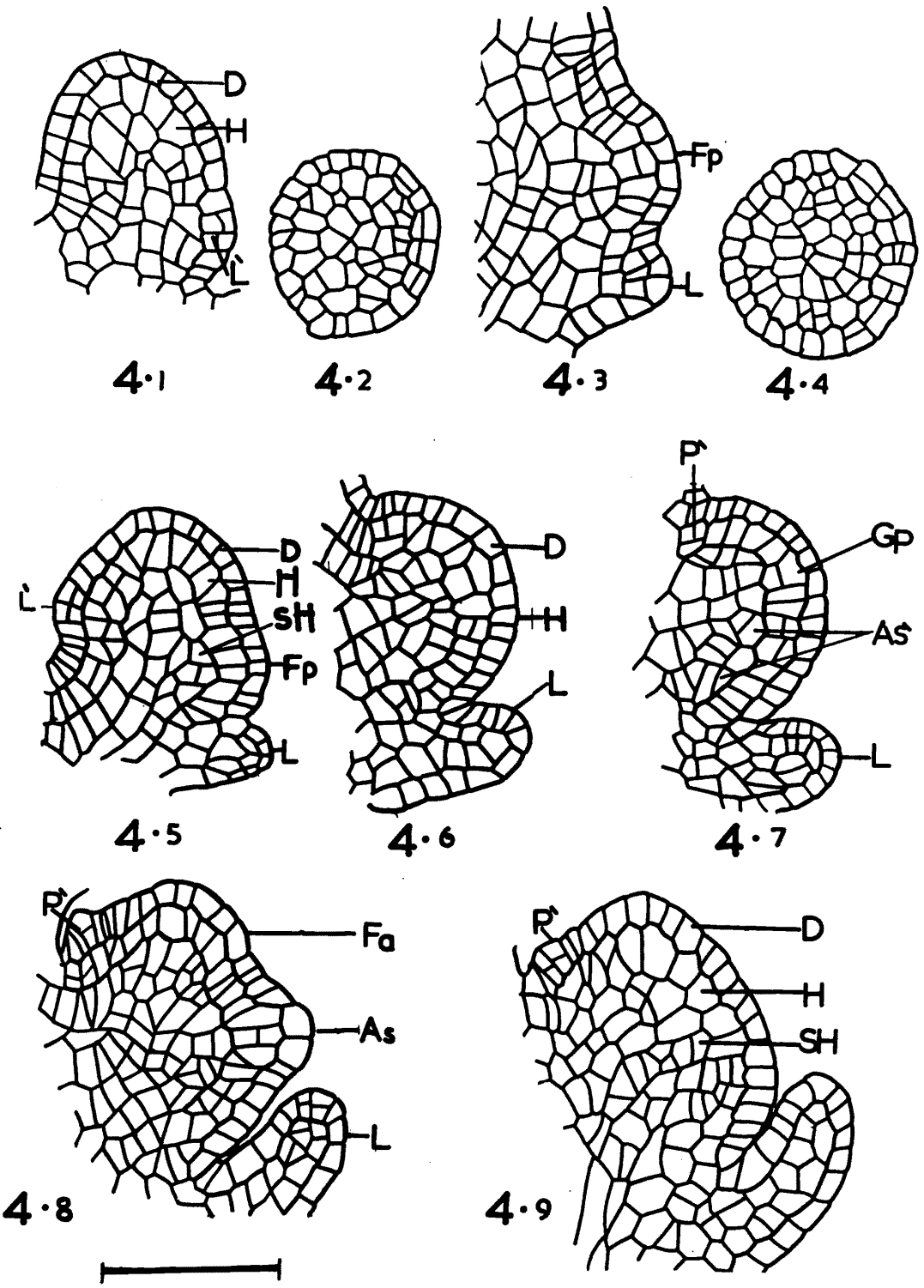

D
H
L
4·1
4·2
Fp
L
4·3
4·4
L
D
H
SH
Fp
L
4·5
D
H
L
4·6
P
Gp
As
L
4·7
R
Fa
As
L
4·8
R
D
H
SH
4·9

Fig4

Prior to the initiation of the floral parts the floret primordium is rounded and has the same characteristic arrangement of cells as occur in the apical meristem of the spikelet and the vegetative shoot, see Fig. 4.6, 4.7, and Plate 8A-C. The dermatogen, hypodermal and subhypodermal layers of the floret primordium derive from the corresponding layers of the axis of the spikelet, whereas in the formation of the core, most of the cells are contributed by the subhypodermis and few by the hypodermis.

(c) Stamen.

Longisections (in plane of glumes), which pass through the centre of the spikelet axis and the apex of the floret primordium, also pass through the centre of the anterior stamen. Such sections, therefore, not only show most clearly the origin of the anterior stamen but also its relation to the origin of other floral parts. For this reason the anterior stamen is taken to illustrate stamen histogenesis, and it is presumed that the origin of lateral stamens is similar.

The stamen originates in essentially the same manner as spikelet and floret primordia. In association with its initiation periclinal divisions in both subhypodermal and hypodermal cells were observed. Whether these occur simultaneously or one after the other, could not be ascertained, as division in one layer without the other is never found in this connection.

Each stamen initates by the periclinal division of usually two to three subhypodermal and one to two hypodermal cells. Figure 4.7 and Plate 8A-C represent the earlier stages in the initiation of the stamen.

As far as could be ascertained, in the light of observations on the early developmental stages, the main bulk of the stamen primordium derives by the repeated periclinal division of subhypodermal cells, whilst the hypodermal cells divide periclinally only once, see Fig. 4.8 and Plate 10A. The cells of the dermatogen layer divide only by the formation of anticlinal walls.

It is most likely that the histogenesis of all the three stamens is simultaneous. The earlier manifestation of the two lateral stamens morphologically is probably due to the difference in the rate of growth rather than in the time of origin.

Later developmental stages of the stamens have not been persued, as these are described in detail by many other workers (Bonnet 1953, 1961). However a few interesting points that occur during its later development were recorded.

The stamens remain more or less sessile even after the differentiation of sporogeneous tissues. The filaments become very much elongated only a little before anthesis.

It was noted, with surprise, that a very small region at the bottom of the stamen gives rise to such a long filament, mostly by cell elongation and only a few cell

divisions, see Plate 9A and B.

Another peculiarity encountered is the presence of conspicuous spaces between the cells of the second, third and fourth layers (subsurface layer and two layers beneath it) of the mature filament, when seen in transection. Plate 9C represents the transection of a filament, where these are shown alternating with the cells of these particular layers. The cells of these layers are also strikingly thick walled.

(d) Palea.

The palea is initiated by the periclinal division of one to three hypodermal cells, followed by similar divisions in the adjacent one to two dermatogen cells. The mode of origin is thus comparable to leaf-like organs.

In Fig. 4.7 Plate 8A - C the palea is being initiated by a periclinal division of one hypodermal cell only; no periclinal division having yet occured in the dermatogen cells. Figure 4.8 and Plate 7C represent a slightly more advanced stage where one dermatogen and four hypodermal cells have undergone periclinal divisions for its initiation.

The palea arises as a narrow ridge of tissues, extending around the adaxial or inner side of the floret primordium.

It is most likely that hypodermal cells undergo only one periclinal division and further development of the palea is by repeated division of dermatogen cells, as depicted by the longisections of floret primordia in Fig. 5.1 and Plate 16A.

Development of the palea is at first more rapid in the two lateral areas than in the mid region. This is evident by comparing Fig. 4.9 and Plate 10B with Fig. 4.8 and Plate 7C. Figure 4.8 and Plate 7C represent the mid-region of the floret primordium where fewer dermatogen and hypodermal cells have undergone periclinal divisions than can be seen to have done so in Fig. 4.9 and Plate 10B, which depict lateral areas. These illustrations are taken from the longisections of the same floret, which are 30 microns apart. As a result, in its early stages the palea assumes a bikeeled structure.

The development of the palea is not followed in transverse sections, because the area of initiation of the palea is so situated on the floret primordium that it is difficult to be sure that the sections are at right angles to the dermatogen and hypodermal layers and this makes it impossible to be convinced as to the real derivation of the various cells seen in "presumed" transverse sections.

In Fig. 9.9 (dp) a few periclinal divisions were observed on the adaxial side of the floret primordium just below the top, perhaps they represent the hypodermal division for the initiation of the palea.

It is impossible so far to say whether the initiation of the palea and stamens is simultaneous or one after the other.

As far as could be ascertained by observations, however, the palea and stamens originate at the same time, because in all longisections so far produced, the first stages of both are present, see Fig. 4.6, 4.7 Plate 8A-C.

(e) Lodicules.

A floret has two lodicules situated on either side of the anterior stamen.

After the initiation of the palea and the stamens have commenced, periclinal divisions occur in the hypodermal cells situated very near the base of the abaxial side of the floret primordium and extend transversely along more or less the whole of the base of the abaxial surface. These probably represent the very first step in the formation of the lodicules. The hypodermal cells situated just below the anterior stamen divide once, while others undergo more periclinal divisions, pushing the adjacent dermatogen cells slightly outward. The periclinal divisions of hypodermal cells are followed or accompanied by similar divisions in one to two adjacent dermatogen cells. These are restricted to the two regions that occur between the primordia of the anterior and lateral stamens.

The pattern of cell divisions for the initiation of the lodicule have been followed in serial longisections of a floret primordium. Plate 10A depicts the median longisection, where the lodicule is being initiated by the periclinal division of about five hypodermal cells, in the

vertical plane, just below the insertion of the anterior stamen. In Plate 10B, that is 40 microns apart from the first section (i.e. 10A) periclinal divisions in subhypodermal cells in addition to those of the hypodermal cells are also observed. These may or may not be associated with the initiation of the lodicule; (see discussion).

Plate 10C, which is 70 microns off the median line of the floret primordium, illustrates a periclinal division in one dermatogen cell in addition to the tier of cells derived by the repeated periclinal divisions of hypodermal cells.

Each lodicule continues its initiation laterally by repeating similar divisions of dermatogen and hypodermal cells as shown in Plate 11A - C.

Although the whole sequence of events was not closely followed, it seems evident that most of the bulk of the lodicules is derived from the dermatogen cells and their derivates and little from hypodermal cells, as depicted by the longisections of the florets in Plate 11C and Plate 12A - C. Plate 13A - C. represents the similar developmental stages of the lodicules in transections.

Course of Vascular System in Mature Lodicules.

A main vascular bundle enters the base of each lodicule. Each vascular bundle divides into three branches, which have been regarded, in the present study, as lodicular traces, and

are shown in Plate 14A at Lotr. In turn, these branches divide several times producing smaller vascular strands that extend from the base almost to the tip of the lodicules.

Plate 14B and C are transections of the floret and the lodicule respectively illustrating the pattern of distribution of the procambial strands in the lodicule.

Plate 15A represents the basal portion of the lodicule in transection, about 170 microns above the level of its insertion on the floret axis. Plate 15B depicts a transverse section of another lodicule about 540 microns below its tip.

In a transection of the basal (or swellon) portion of a mature lodicule, the vascular strands are fewer in number and bigger in cross section than the ones in the upper (or marginal) portion. Each vascular strand in the basal part consists of both xylem and phloem elements, surrounded by rather narrower parenchymatous cells, giving the strand a more or less circular shape, though a regular sheath-like layer is lacking in most of them in this region, see Plate 15A and a. However, higher up in its course each bundle has a definite sheath, Plate 15B and b.

The vascular elements decrease in number as the strand runs from below upwards, so that the terminal part of a vascular strand is usually composed of a single vascular element surrounded by four to six parenchymatous cells, which are narrower in diameter and arranged in the form of a sheath. In some cases the vascular elements may vary from

two to four. These vascular elements in the terminal ends of the strands are probably xylem (vessels or tracheids) rather than phloem elements.

The vascular strands, which are nearer to the inner surface (next to the carpel) of the lodicule, tend to have more vascular elements than the rest, as may be noted in Plates 14B and 15A and B.

(f) Carpel.

(i) Ovary.

The apex of the floret primordium is rounded at the time of carpel initiation.

In a longisection (in the plane of glumes) the beginning of the carpel is detectable on the anterior side of the floret axis, after the primordium of the anterior stamen has developed as a small papilla.

The earliest visible indication of its initiation is the periclinal division of two to three hypodermal cells, situated just above the point of insertion of the anterior stamen and only a few cells away from the growing point of the floret axis. In some cases these cells may divide at an angle as indicated in Fig. 5.1 and Plate 16A at C', instead of truly periclinally. The periclinal divisions in the hypodermal cells are followed in one to two adjacent dermatogen cells by similar divisions, see Fig. 5.1 and Plate 16A at C'. Soon after, more dermatogen cells above and below these undergo periclinal divisions as indicated in Fig. 5.2.

Figure 5.

Figs. 5.1 - 5.6. Longisections of young florets illustrating the stages in the development of the carpel.

Figs. 5.1 & 5.2. Showing initial stages of carpel at C'.

Figs. 5.3 & 5.4. Slightly later stages when the carpel primordium has spread round the axis to appear at C".

Figs. 5.5. &5.6. Still later stages, C' indicates the anterior portion and C" the posterior portion.

Note:- Each scale is equal to 100 micron the bigger is for detailed drawings and smaller for outline ones.

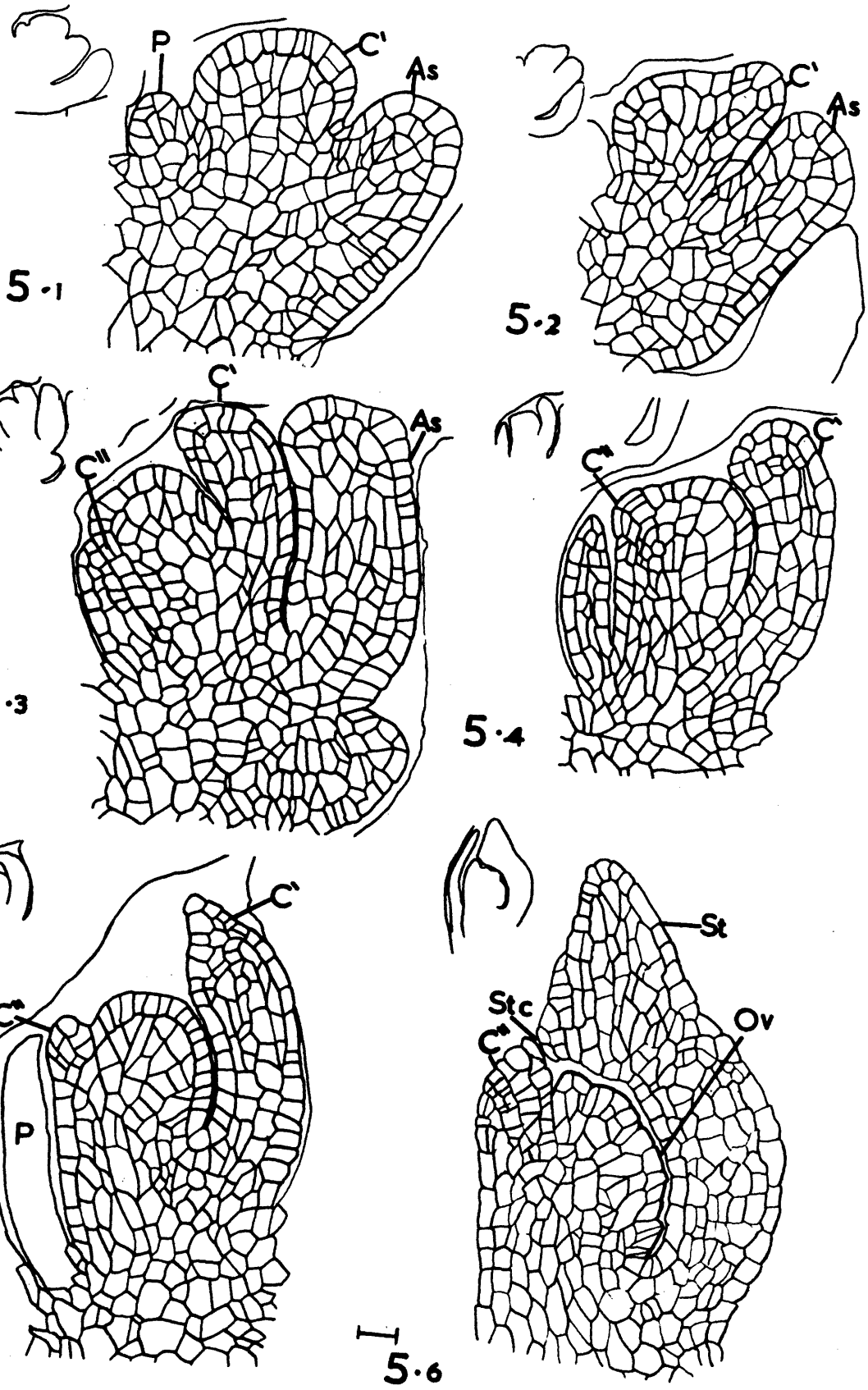


Fig5

Figures 6.1, 6.2, and 6.7, are transections of floret apices indicating more or less similar initial stages of the carpel.

The initiation of the carpel extends circumferentially around the floret apex by periclinal divisions in the dermatogen and hypodermal layers. Before completion of this lateral growth, the small crescent shaped protrusion formed by the first divisions begins,to grow upwards. Thus apical growth and lateral expansion combine to produce a structure that is highest at the point of its origin and that slopes down along the margins, which are in process of encircling the floret axis, see Fig. 5.3. A corresponding morphological stage is indicated in Plate 3A at C. The two margins advancing towards each other first meet and ultimately fuse, with the result that the floret apex, is completely encircled as seen in Figs. 5.3 to 5.6 and Plate 16 B and C.

The encircling growth of the carpel is not horizontal, but follows a gradually rising course, so that its insertion on the floret apex is at a slant.

Figures 6.3 to 6.10 represent similar stages in the progress of carpel initiation as seen in transections. Plate 3B and C at C depict a corresponding morphological stage.

The carpel emerges as a cowl shaped structure because of earlier initiation and more rapid growth of the anterior than the posterior side. Soon after, the rate of

Figure 6.

Figs. 6.1 - 6.10, are transections of young florets illustrating the initiation and various stages in the development of the carpel.

Figs. 6.1 - 6.3, are serial sections from a floret showing the initiation of the carpel on the anterior side of the floret axis at C'.

Figs. 6.4 & 6.5, transection of another floret where the carpel is being initiated on lateral sides as well (C" and C').

Figs. 6.6. - 6.8, transections from three different florets indicating similar stages in the initiation of the carpel at C' and C".

Figs. 6.9 & 6.10, transections from slightly older florets probably corresponding to 5.5. and 5.6, respectively, where the carpel is being initiated on the posterior side as well as by the periclinal divisions of dermatogen and hypodermal cells (C").

Note:- Due to the difficulty in orientating young spikelets and florets, transections exactly in horizontal plane could not be obtained, hence the initiation of the carpel can only be shown by small patches of dividing cells.

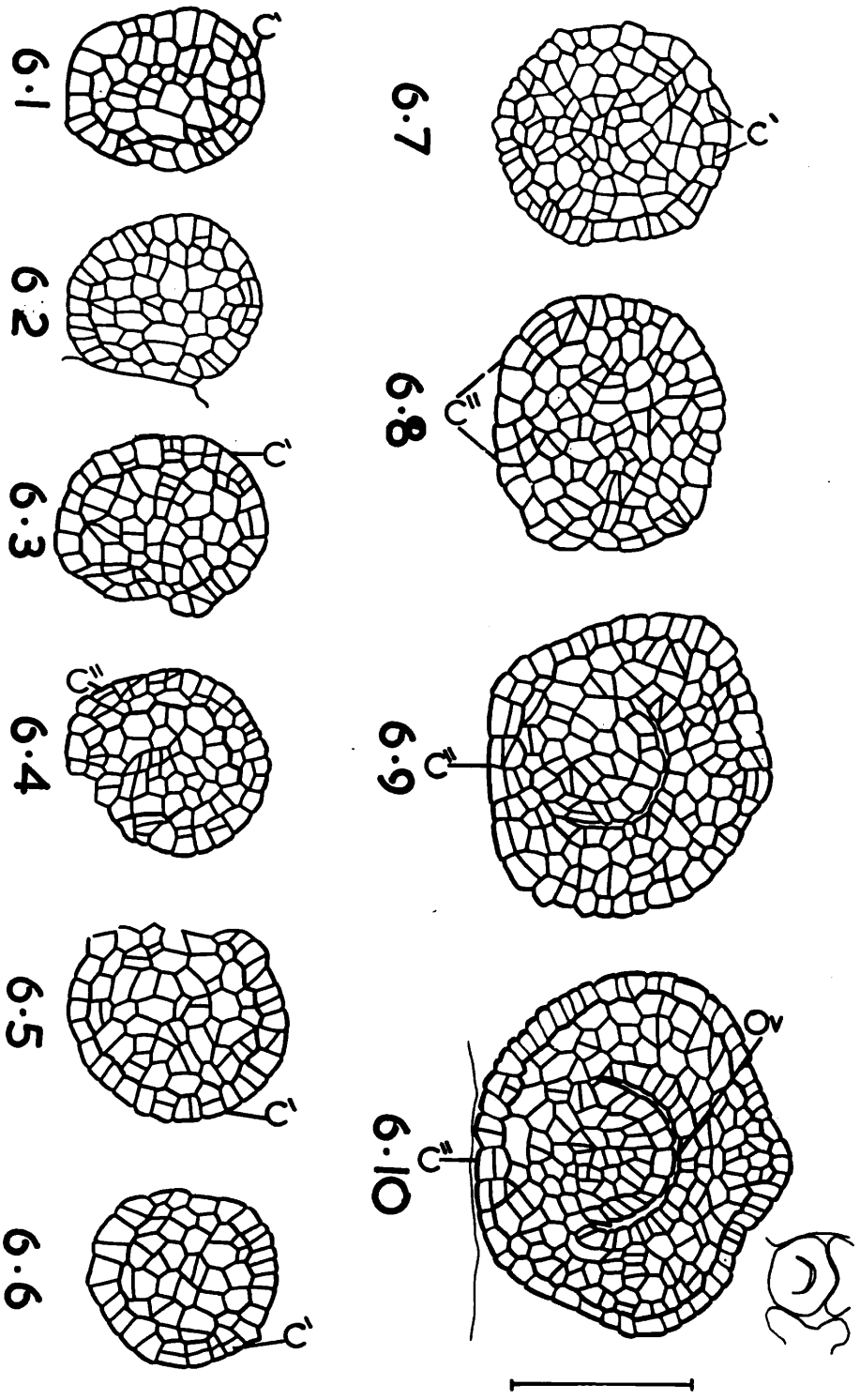


Fig6

growth of the posterior side accelerates and by repeated divisions of dermatogen cells and possibly by a few divisions of hypodermal cells, is also raised up. Thus, although the anterior side of the carpel wall is still higher a complete ring has formed enclosing the loculus of the ovary. This is indicated in Figure 5.6 and Plate 16C. The carpel in Plate 3D represents morphologically a similar stage. The opening at the top of the loculus of the ovary gradually narrows down by marginal growth on the inner face of the ovary wall, see Plate 17C, although it never completely closes, but persists as a "styler canal" in the mature pistil. Plates 17B and C and 19D illustrate its presence in young as well as in fairly mature carpels.

It was impossible to be quite sure whether the entire bulk of the carpel derives from the dermatogen cells alone or from both dermatogen and hypodermis. As far as could be ascertained by observations on the early development of the carpel, the dermatogen cells and their derivatives contribute most to the carpellary tissues, as indicated in Plate 17A at C which is a longisection of a young carpel, (at right L to the plane of the glumes). At least its posterior portion derives mostly from the repeated divisions of dermatogen cells as depicted in Fig. 5.6, Plate 16C at C".

(ii) <u>Stigma</u>.

Prior to the initiation of the stigmas the growth

of the carpel is uniform all round its margin (i.e. at the free edge of the cowl). Soon the margins of the carpel opposite to the laterally placed stamens grow more rapidly than the other portions, giving rise to the two primordia of the stigmas. This pattern of development places the two stigmas on the anterior half of the carpel. Shortly, after their initiation each stigma is cone shaped and hence appears more or less triangular in longisection, as can be noted in Fig. 5.6 and Plate 16C. Each stigma grows in length and diameter by anticlinal and periclinal divisions of both dermatogen and hypodermal cells, see Plate 16C, but in the later stages of development, most of the cells divide parallel to the long axis of the stigma, as may be noted in Plate 17B. Therefore, at maturity the cells are long and narrow. In transection each stigma appears to have two specialised patches of tissue (Plate 20C). One, the vascular bundle, is situated near the outer surface and runs parallel to it throughout its length, except at the extreme tip, where it seems to be absent. The other, which occurs near the inner surface is stigmoid tissue, which also runs the whole length of the stigma.

Besides the two laterally placed stigmas a projection of the tissues is regularly observed on the side of the carpel next to the palea (i.e. the posterior side). Occasionally another projection is seen on the anterior side.

The posterior projection is a small protrusion measuring about 100 microns in height, broader at its base

but rather sharply tapering upwards. In the material studied, unlike the two lateral stigmas, the posterior projection has no definite single epidermal layer. However, it becomes evident lower down, as can be seen in Plate 18D. The organisation of the cells in the projection is also peculiar, as should be noted in Plate 18 A-D. No trace of vascular supply is found in it. (Most likely the posterior projection also bears a few hairs, which are similar to carpellary hairs. This is assumed because of the presence of larger thick-walled cells in the peripheral region of this projection).

The anterior projection has been regarded as an anterior stigma as it is surrounded by a definite single epidermal layer, and in addition some of the cells of the outer layer enlarge and even divide, as may be noted in Plate 19 A and B, suggesting the initiation of stigmatic hairs. The anterior projection is slightly longer than the posterior one measuring about 120 microns in length, no trace of vascular elements is detected in this either.

In one instance, the presence of a posterior and an anterior projection has been observed in the same carpel. The posterior one is inserted higher than the anterior, the difference in their levels being 80 microns.

Initiation and development of stigmatic hairs.

The tips, the inner, and part of the lateral surfaces of a mature stigma are densely covered almost to the base, with numerous multicellular hairs of a very characteristic

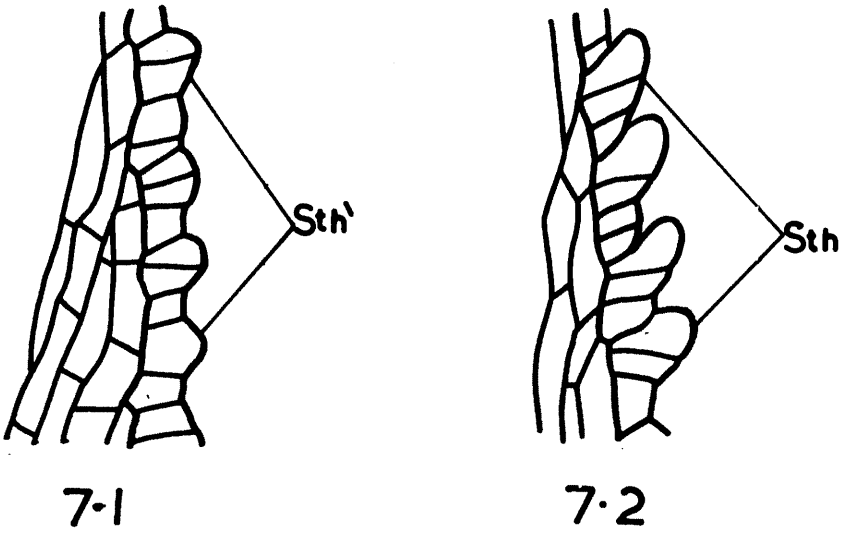
structure. Most of the exterior surface of the stigmas is without stigmatic hairs. Each of the hairs is filament like, and composed of four rows of elongated cells.

It is observed, in longitudinal section of stigma, that each stigmatic hair arises from a single epidermal cell, which occurs alternately in a longitudinal row, as indicated in Fig. 7.1. The cell divides by a wall which is not quite at right angles to the surface of the stigma, but rather oblique, with the result that two narrow cells are formed, one lying above the other as shown in Fig. 7.1. The acropetal member of the pair elongates and protrudes outwards and upwards, see Fig. 7.2. Soon afterwards this cell divides by two vertical walls that are at right angles to one another, and perpendicular to the surface of the stigma, so forming a group of four cells, as can be noted in Fig. 7.3 (one dividing wall cannot be seen, as it is parallel to the plane of the section). Plate 20A and B represent similar initial stages in transections.

The four cells so formed behave as a kind of apex and by growth and repeated transverse divisions produce a hair composed of four rows of cells, see Fig. 7.4. Eventually each cell of the hair forms a small protuberance at its distal end, giving the whole hair its final papillate appearance. Plate 20C depicts the mature stigmas and stigmatic hairs in transection.

Figure 7.

Figs. 7.1 - 7.4. Longisections of young stigmas showing various stages in the development of stigmatic hairs (Sth).



7·1 7·2

7·3 7·4

100 µ

Fig7

(iii) (a) Ovule.

After the differentiation of the carpel the floret apex slightly increases in size and organises itself again into its three germ layers, i.e. dermatogen, hypodermis and subhypodermis, although in some cases the subhypodermis is not clearly distinguishable as indicated in Figs. 5.3 and 5.4.

In the beginning, the developing ovule (i.e. the floret apex now enclosed in the carpel) is directed vertically upwards Figs. 5.3 and 5.4, but gradually it starts bending over towards the median line of the carpel. Firstly, it assumes a horizontal position, pointing outwards, and then eventually downwards. This change in orientation is mainly caused by the greater elongation of the cells on the posterior side of the floret, rather than by a difference in the rate of cell divisions, as can be noted in Figs. 5.5, 5.6 and Plates 16B and C.

The archesporial cell makes its appearance very early in the development of ovule. It is derived from a hypodermal cell.

(b) Integuments.

The two integuments are initiated soon after the developing ovule begins to bend over, Figs. 8.2 and 8.3, and their primordia are easily recognisable by the time the ovule is more or less horizontal, see Fig. 8.4 Plate 21A. In both

longitudinal and transections of carpel, although periclinal or oblique walls may be observed in one or two hypodermal cells just before or immediately following initiation, as may be noted in Figs. 8.2, 8.9, 8.10 and 8.11, most if not all of the actual integument seems to be derived from the dermatogen layer alone, see Figs. 8.4, 8.5, and Plate 21B. Some of the dermatogen cells involved in the initiation of an integument have oblique new walls rather than strictly periclinal ones, as seen in Figs. 8.2, 8.3, 8.5, and Plate 21B.

The initiation of the integuments (at least in the material studied) presents two rather surprising features. Firstly, both integuments commence their initiation on the same side of the ovule, that is appearing on the upper side, in a longitudinal section of the carpel. This is before the growth spreads round and is seen on the lower side as illustrated in Fig. 8.3 and Plate 21A. Later they continue their initiation circumferentially around the ovule by similar divisions, thus encircling the developing ovule completely as depicted in the longisections of the carpels in Figs. 8.4, 8.5, and Plate 21B. This indicates that integuments are not only similar to foliar structures in their initiation but also in their early circumferential development.

Secondly, the inner integument seems to be initiated before the outer one, see Figs. 8.2 to 8.5. In all cases examined so far the same course of events was observed.

It was expected that the outer integument would initiate earlier than the inner, and that they would alternate in their positions, so completing the disticheous arrangement of the leaf-like structures on the floret axis.

However, present observations do not conform with these expectations and indicate that the initiation of the inner integument is followed by the outer and both integuments originate on the same side of the floret axis. In Fig. 8.2 the initiation of the "first" integument is indicated by the division of a dermatogen cell. This divided cell is not occupying the same position on the circumferance of the ovule, as the outer integument has at a slightly later stage when both integuments are present, see Fig. 8.4 and 8.5. Furthermore in the early stages of development, the inner integument is always more advanced than the outer, as depicted by Figs. 8.3 to 8.5 and Plate 21A and B. In addition, in a transection of the carpel, the first initiated (or inner) integument is closer to the dome of the ovule, than the elongated cells which are discernible further away and which most probably constitute the seat of origin of the other (or outer) integument, see Figs. 8.11, 8.12 and Plate 21C.

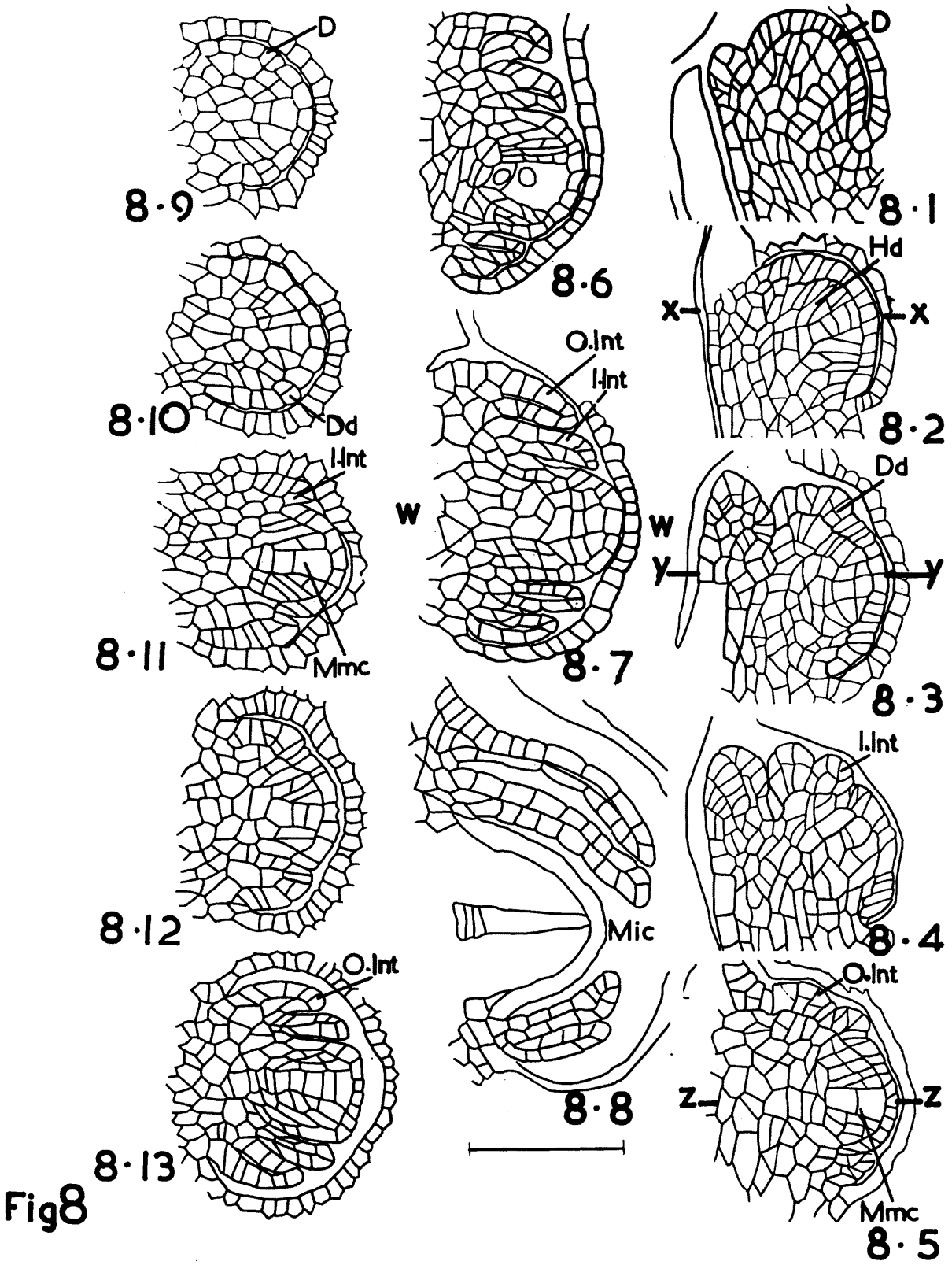
Figures 8.9 to 8.13, illustrate the initiation of the integuments as seen in transections of young carpels. Figures 8.9 and 8.10 depict sections taken at right angles to that shown in Fig. 8.2 in the plane of X-X, the stage of development being about the same. Similarly 8.11, 8.12 and

Figure 8.

Figs. 8.1 - 8.8. Longisections of carpels, illustrating different stages in the development of integuments.

Figs. 8.9 & 8.10. Transection of a carpel, similar to that shown in Fig. 8.2, taken in the plane of x-x.

Fig. 8.11. represents 8.3. in transection, cut in the plane of y-y, similarly 8.12 is of Fig. 8.5, in the plane of z-z, and 8.13 is comparable to that of 8.7, taken in the plane of w-w.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8·6
D
8·1
Hd
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x
8·10
Dd
O.Int
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Dd
y
y
8·11
Mmc
8·7
8·3
I.Int
8·12
Mic
8·4
O.Int
O.Int
z
z
8·13
8·8
Mmc
8·5

Fig8

8.13 represent 8.3, 8.5 and 8.7 in transections in the plane of Y-Y, Z-Z and W-W respectively.

The configuration of the cells, as seen in Figs. 8.6, 8.8, and 8.13, suggests that both integuments continue growth at their margins for a considerable time.

The micropyle is formed by the inner integument only. The cells of the third and fourth layers back from the micropyle get bigger than those of the first and second layers, so forming a slightly raised ring. The margin of the outer integument does not grow as far as that of the inner, but terminates at the level of the ring formed by the inner integument, as may be noted in Plate 21D.

No trace of any vascular supply could be detected anywhere in either of the integuments.

Differentiation of vascular strands in stamens and carpel.

I. Procambium*.

In spite of the fact that an extensive literature is available on the developmental anatomy of monocotyledons, it contains very little information about the position of origin and direction of differentiation of the procambium (or provascular strands) and first vascular elements.

There is a divergence of opinion with respect to the characteristics which can be ascribed to the cells initiating a procambial strand. However, most workers agree that elongated darkly staining cells, dividing parallel to the long axis of that particular organ, can be identified as precursors of a procambium; as Tepfer (1953) states; "The criteria generally cited are darker staining reactions, elongation, and division in a plane parallel to the long axis". According to Esau (1954) also; "Commonly the youngest procambium is identified by the shape of cells and by their position with respect to the vascular tissue in the mature shoot. When elongated cells appear in the proper position for the prospective vascular tissue, the cells are interpreted as procambial. Usually these cells also stain densely, although the procambium of older plant parts may be distinctly vacuolated. The density of the cytoplasm may be

*Foot Note:- Following Esau (1943) the word procambium has been used for the precursor ofavascular strands.

visible before the cells assume the elongated shape. Sometimes the stainability of cytoplasm increases when the tissue differentiates into procambium. Buvat (1951b) interprets such a change as a cytologic differentiation, involving a decrease in size of vacoules and reduction in size and number of plastids that may be present".

In the present study, most of the above criteria were considered, while tracing the procambial strands both in stamens and carpel. Although in very early stages of initiation no marked distinction was observed in the density of cytoplasm, the procambial cells could be distinguished from their neighbours by slight differences in their staining. Later on in their development they were distinguished by their characteristic arrangement and position.

(a) Stamens.

By the time the stamen is about 420 microns high, including its small filament, it has a fairly well developed procambial strand, which already has its first sieve element mature (Fig. 12.1 - 12.5). Plate 30 shows the morphological appearance at about this stage.

Serial transections of younger florets were examined to determine the initial stages of a procambial strand in the stamen. It is observed, that in each stamen, the procambial strand appears in isolation slightly above the middle region, and is not the mere upward continuation of a more mature

strand in the axis. The earliest indication of the initiation of a procambial strand is the longitudinal division (parallel to the long axis of stamen) of two to three cells about half way up the stamen. This isolated patch of narrow and most probably elongated cells may sometimes be distinguished from neighbouring cells by its slightly denser cytoplasm and difference in staining in microscopic preparations. However, in most cases (of the material studied) the cytoplasm of adjacent parenchymat◆ous cells is as dense as that of procambial cells, and it is rather difficult to differentiate one from the other on this basis. Mostly the initial stages of the procambium are distinguished by the characteristic arrangement of the cells and their position in the stamen.

Most likely the presence of denser cytoplasm in the parenchyma cells surrounding the procambium indicates their meristematic nature.

In the material studied, the initial stage of the procambium was found represented by a few periclinally dividing cells about 28 microns below the top of the stamen, and ⁑⁑ extended for about 42 microns in length. The young stamen by this time was about 105 microns in height, and was almost sessile.

Once the procambial strands are initiated their differentiation is very rapid.

Figures 9.1 to 9.3 and Plate 22A - D, are taken from the serial transections of young florets, depicting the

position of origin of a procambial strand in a lateral stamen. In Fig. 9.1 and Plate 22 A the procambial strand is represented by the periclinal division of a single cell, while a very well outlined procambial strand is present at the level shown in Fig. 9.2 and Plate 22B at Pcst, which is only 21 microns lower down. It is not detectable again, where the stamen joins the floret axis, as indicated in Fig. 9.3 and Plate 22C, which are taken from sections 42 microns still further down. Plate 22D depicts the very base of the same lateral stamen at Ls, where there is no suggestion of the presence of a procambial strand.

Figures 9.2. to 9.4 illustrate similar stages for the position of origin of the procambial strand in the anterior stamen. The illustrations are taken from three sections, which are 42 and 28 microns apart respectively.

The above illustrations, depicting the position of origin of procambial strands in both lateral and anterior stamens, also lend support to the conception of bipolar differentiation of the procambial strands. Figure 9.2 and Plate 22B indicate more or less the middle regions (50 Microns below the top) of the stamens, where the procambial strand is composed of eight or nine cells, while higher up or lower down (see Fig. 9.1 and 9.3 and Plate 22A and 22C) it is represented either by a division of a single cell or even is not present at all. Thus it is logical to conclude that the procambial strand initiates in the stamen itself and then

Figure 9.

Figs. 9.1 - 9.4. Serial transections of a developing floret, illustrating the position of origin of the procambial strands in lateral (Ls) and anterior stamen (As).

Figs. 9.5 & 9.8. Longisections (at right L to the plane of glumes) of the carpels.

9.5. dermatogen cells of the future ovule divides periclinally, initiating the integument.

9.6. shows tier of cells derived from the longitudinal divisions of dermatogen cells for closing the top of the ovary, and also illustrates the pattern of development of two stigmas.

Figs. 9.7 & 9.8. Periclinal divisions in the dermatogen and hypodermal cells initiating the lodicules (Lo).

Fig. 9.9. Transection of a young floret, periclinal divisions in the hypodermal cells (dp), possibly are concerned with the initiation of palea.

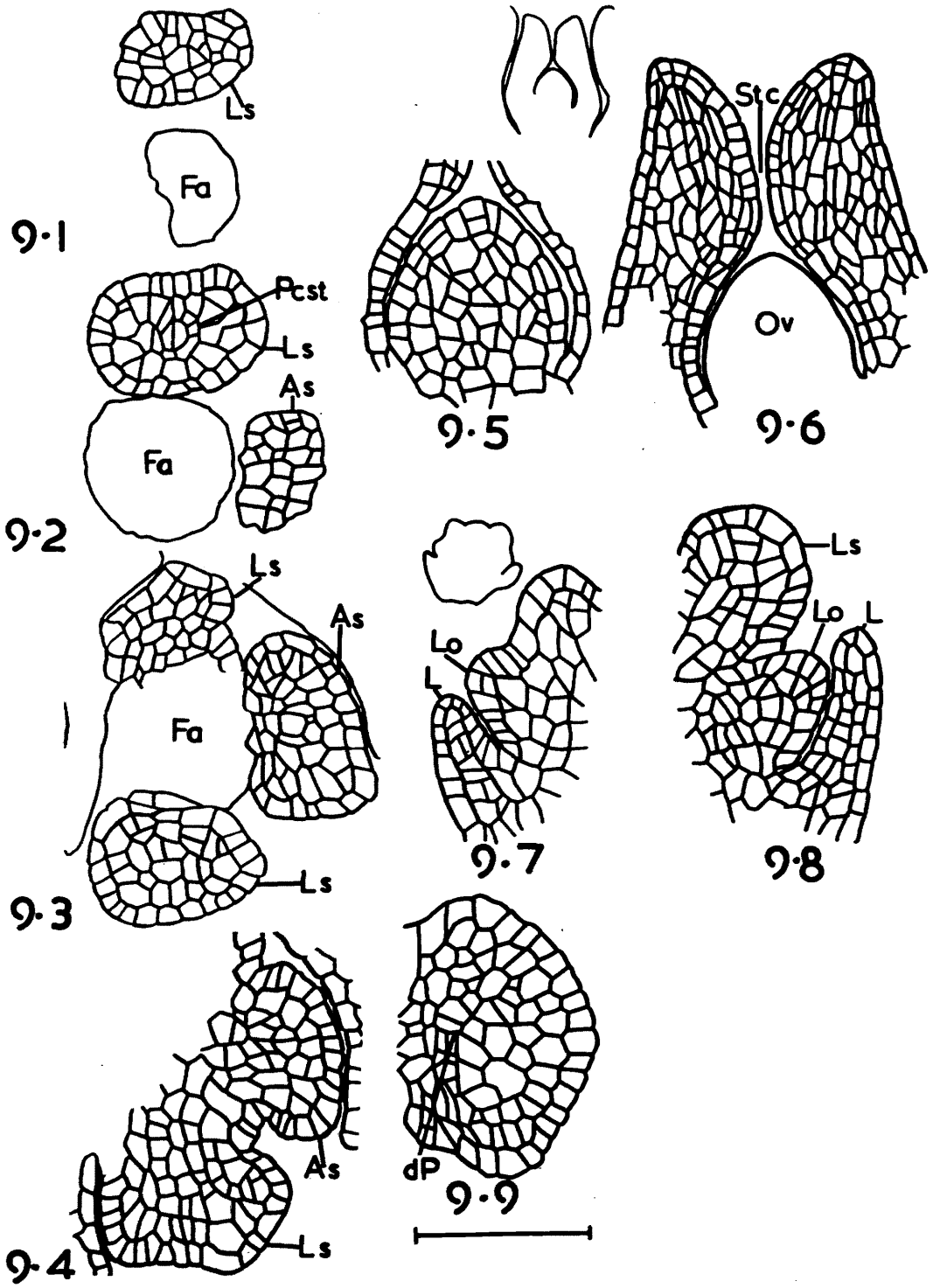
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Fig9

differentiates both acropetally and basipetally.

The early divisions initiating a procambial strand appear to spread from the centre of the future strand, as may be noted in Fig. 9.1 and Plate 22A. Later the cells around the central group of cells (formed by former divisions) have a tendency to divide and to elongate tangentially with respect to this group, see Fig. 9.2 and Plate 22B. Since these early divisions are followed by only a little cell enlargement, the newly formed procambial cells are relatively smaller than the neighbouring cells.

(b) Carpel.

(i) Median and lateral procambial strands.

In a carpel (at about the stage shown in Plate 3E) there are only three provascular or procambial strands, a median and two laterals. Each lateral supplies the stigma on its side of the carpel, whilst the median one ends in the carpel wall just below the top.

An attempt was made to determine whether the three strands seen in the young carpel are merely the upwardly propagating ends of more mature strands in the axis, or if they originate independently of the axial supply and connect up with this later.

Careful examination of serial sections left no doubt that all three carpellary procambial strands originate in the carpel itself. Representative transverse sections to illustrate this are shown in Figs. 10.1 to 10.17 and Plate 24A - D.

The earliest stage observed in the initiation of a procambial strand is longitudinal division (parallel to long axis of carpel) of a small group of cells (three to four), just above the middle region of the carpel. From this region the strands then differentiates acropetally and basipetally by similar divisions.

The median procambial strand appears earlier than the two laterals.

A transection of the upper part of the ovary wall is shown in Fig. 10.9 with no suggestion of the presence of the median procambial strand in it. A section cut 14 microns lower down shows the presence of a distinct strand, and this can still be detected in Fig. 10.11, which is about 63 microns further down. However, the procambial strand is represented only by periclinal divisions of few cells in the base of carpel at about the level shown in Fig. 10.12 which is 56 microns still further down.

In this particular material, the median procambial strand was observed 21 microns below the top of the ovary wall and it extended for about 147 microns in length. By this time the carpel was 210 microns high.

Figures 10.13 to 10.17 illustrate the position of origin of the median procambial strand in another carpel, cut at intervals of 21, 49, 63 and 42 microns. Plate 24B-D shows the same features in both the median and lateral strands of yet another carpel. These illustrations are

taken from three sections, which are 70 and 105 microns apart respectively. A still younger stage has been detected in other material, where the median procambial strand appeared in the anterior portion of the carpel primordium, before this has completed the encircling of the floret apex, and when its median portion is only about 132 microns high. It appeared 60 microns below the top of the ovary wall, and extended for about 48 microns in length. In another carpel of similar age, with its medium portion 161 microns high, the presence of a median procambial strand has been detected, which extended for about 98 microns in length. It is represented 28 microns below the top of the ovary wall by a division of a single cell. This division is periclinal, parallel to the outer surface of the carpel. Lower down at the level shown in Plate 23A, certain adjacent cells have also divided or are about to divide around the periphery of the small group of cells, which has formed by previous divisions. Thus, the strand is composed of six to eight cells and is easily distinguishable. There is, however, no trace of a procambial strand at about the level depicted in Plate 23B which is 24 microns further down.

The earlier stages of initiation of the lateral procambial strand are recorded from a carpel that measures about 294 microns in length.

Figures 10.1 to 10.4, illustrate in transection the stigma and the region of the carpel wall below it.

No strand is detectable in the section used for Fig. 10.1, but a strand is quite distinguishable only 14 microns lower down, Fig. 10.2. This strand could be traced in the carpel wall down to the level shown in Fig. 10.3, which is taken 91 microns below the previous one. However, no trace of it could be found at a level 98 microns below this, as may be noted in Fig. 10.4.

Figures 10.5 to 10.8 illustrate a similar series of transections of a carpel from another floret, taken at intervals of about 49, 71 and 154 microns respectively. The position of initiation and direction of differentiation of lateral procambial strand is also illustrated in Plate 24A, C-D.

By comparing the observations on the origin of the median procambial strand in a younger (Plate 23A and B) and a slightly more advanced carpel (Fig. 10.9 to 10.12 and Fig. 10.13 to 10.17), it is tentatively concluded that the strand initiates as an isolated patch of tissue at about the middle region of the carpel and then differentiates both acropetally in the organ itself and basipetally to join the mature strand below, in the axis.

Although no earlier stages than these (Figs. 10.1 to 10.4 and 10.5 to 10.8) could be found in the initiation of the lateral procambial strand, however, in the light of observations of its position of origin, and its discontinuity

Figure 10.

Figs. 10.1 - 10.17. Transections of developing carpels, illustrating the position of origin of the median and lateral procambial strands.

Figures 10.1 - 10.4 and 10.5 - 10.8, transections, cut at a series of levels, of the lateral procambial strands of two different florets.

Figures 10.9 - 10.12, position of origin of median procambial strand.

Figures 10.13 - 10.17, the same from a different floret.

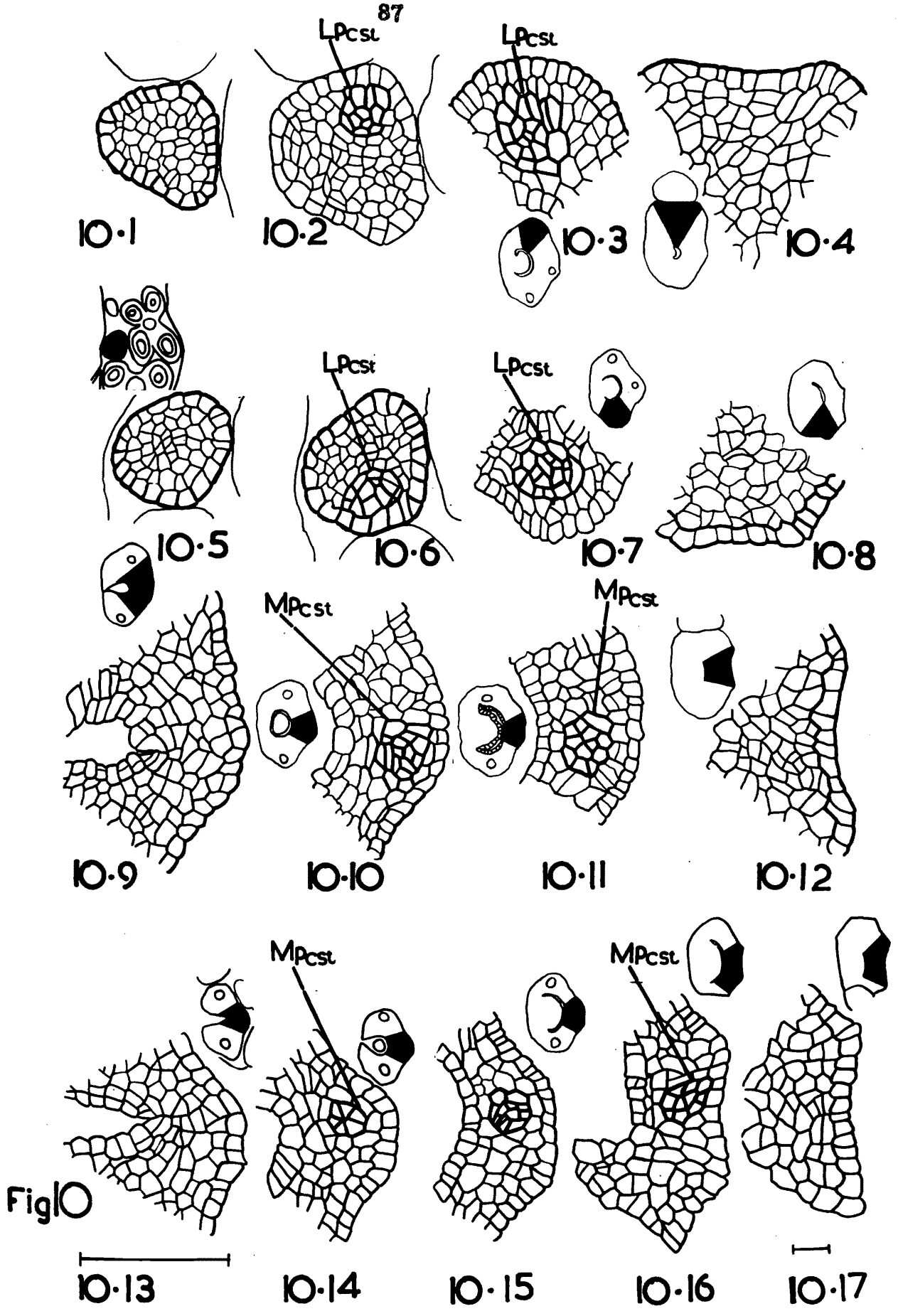
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Fig10

above and below, the same bipolar differentiation may be concluded.

After the narrow elongated cells of the procambium have organized into a strand, the latter increases in diameter mainly by the division of cells within it, while the addition of cells may also occur on the periphery of the strand. These divisions are followed by little enlargement, so that resulting procambial cells remain small in their early stages of development.

(ii) Funicular procambial strand.

The strand that appears in the posterior wall (i.e. next to the palea) of the carpel is known as the funicular (or placental) strand. In the present study this portion of the carpel has been regarded as axial in nature, (see discussion).

The procambium of the funicular strand differentiates much later than that of the median and lateral procambial strands. At the time of initiation of these three strands the funicular procambium is represented by a few dividing and very small cells at the very base of the carpel.

Careful examination of serial transections of more mature carpels left no doubt that the procambium of the funicular strand differentiates acropetally i.e. it is the upwardly propagating ends of more mature strands in the axis.

Representative serial transections of a carpel are illustrated in Figs. 11.1 to 11.4. Figure 11.1 depicts the

Figure 11.

Figures 11.1 - 11.4, serial transections of a carpel illustrating the acropetal differentiation of the funicular procambial strand and the vascular elements in it.

Fig. 11. 1, terminal portion of the funicular procambial strand with no vascular elements in it (Fpost) (cut at the level of the base of ovary loculus).

Fig. 11.2. Median and lateral procambial strands with very well differentiated phloem elements, while funicular procambium does not show any.

Fig. 11.3. Vascular cylinder and median strand (before the latter joins the former).

Fig. 11.4. Vascular cylinder at the base of the carpel.

Note:- The small circles having been drawn in the phloem cells are not meant to indicate the presence of nuclei in them, but just to distinguish these from the surrounding cells.

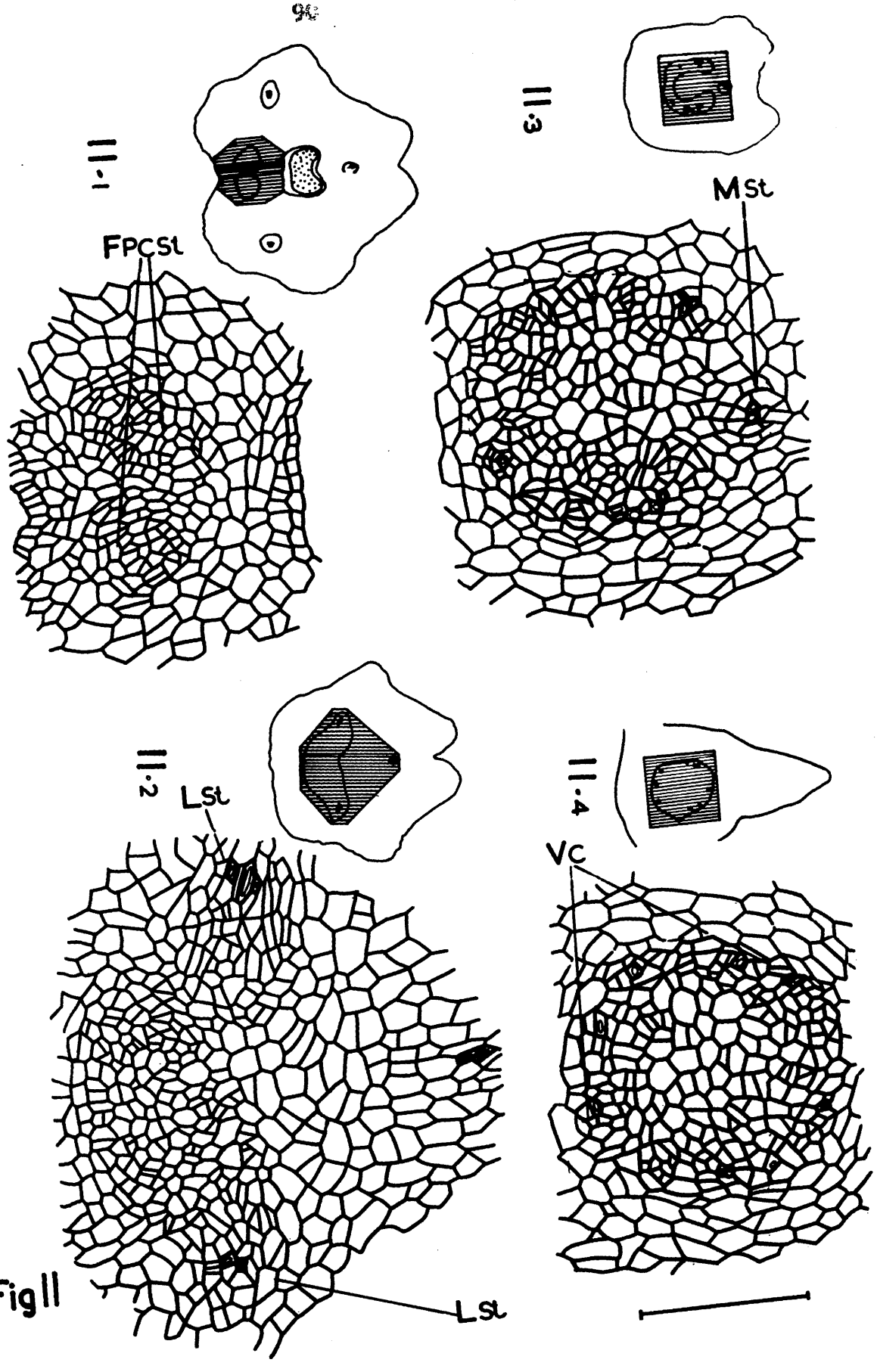


Fig II

region of the posterior wall, at the level of the base of the ovary loculus. At this level the funicular strand seems to be composed of two groups of cells. Figures 11.2 and 11.3 illustrate the regions lower down and show in addition to the funicular strand, the descending two laterals and the median strand just before they join the vascular cylinder. This is probably mainly formed by the union of these four strands and is present at the base of the carpel, see Fig. 11.4.

The funicular strand like the others increases in its diameter by further divisions within the strand and also by addition of cells on its periphery. The divisions within the strand are not always periclinally orientated, with respect to the outer surface of the strand, they may be oblique or some times irregular as may be noted in Figs. 11.1 and 11.2.

The cells initiating the procambium of the funicular strand, can easily be distinguished from neighbouring cells by their denser cell contents and much smaller diameter.

Differentiation of vascular strands in stamens and carpel.

II. First elements of Protophloem and Protoxylem.

(a) Stamens.

(i) Course of the vascular strand in a mature stamen.

The floret has three stamens, one is anterior in position while two are laterally placed. Each stamen consists

of a rather large four locular anther borneupon a filament. A single collateral vascular bundle, that is one composed of both phloem and xylem elements, extends throughout the length of filament and the connective of the anther.

(ii) Protophloem.

After the procambium has been organized as a strand of narrow elongated cells, further increase in the thickness is mainly due to cell divisions within the strands. When first differentiated the cells of the procambial strand can only be distinguished by their small diameter and by their position, from the adjacent parenchyma and not by the density of their cytoplasm. By the time the first vascular element (i.e. protophloem element)differentiates the procambium appears more distinct perhaps due to the progressive vacuolation of the surrounding parenchymatous cells.

Shortly after the procambial strand is delimited, a procambial cell near the outer periphery of the strand (that is one on the side of the strand, nearest to the outside of the florets) starts showing the thickening, characteristic of a sieve element.

The initiation of the protophloem of a stamen is relatively early, in comparison with that of a carpel.

Examination of serial transections of young florets suggests that the first phloem element (one in transection) originates in isolation at about the middle region in the

anterior stamen. The same is most likely true for the two lateral stamens also.

The earliest visible differentiation of the sieve element is the change in appearance of the cell wall. The cell can be picked out from its neighbours by its thicker, deeply stained walls, although the cytoplasmic contents are still as dense as in the other procambial cells. The thickening of the cell wall is more evident, at first, at the cell corners. The mature sieve elements become much more conspicuous, due to their thickened walls and lack of stainable cell contents, as may be noted in Figs. 12.2 to 12.4 and 12.7 to 12.9 and Plate 28B.

The earliest stages in the origin of protophloem (first sieve element) were observed in a stamen, which was about 252 microns high (including its filament that measured 72 microns in length). The element was detectable about 72 microns below the top of the stamen and extended for about 96 microns in length. Probably it was composed of two sieve elements, arranged end to end in a single row, (Plate 26A-C).

Figures 12.1 to 12.5 and Plate 27A - C are serial transections of a young floret illustrating slightly later stages in the differentiation of protophloem in an anterior stamen. In Fig. 12.1 there is no suggestion of the presence of a sieve element, whereas in a section cut 126 microns below, which is represented in Fig. 12.2, it can be very well picked up by its thickened and deeply stained walls.

It can still be traced in Figs. 12.3 and 12.4, which are 91 and 63 microns respectively, further down. However, no sieve element is detected in the base of the filament, about 133 microns still further down, as may be noted in Fig. 12.5.

Plates 26 and 27B, each depict an immature sieve element. It should be noted, though its walls are already somewhat thickened and deeply stained, that the cytoplasmic contents are still as dense as in the procambial cells. Plate 28B illustrates mature sieve elements, which are very conspicuous due to their evenly thickened cell walls and enucleate condition.

From the pattern of their arrangement in later stages, it is obvious that the additional protophloem sieve elements differentiate centripetally from the first. Eventually a cluster of sieve elements is formed near the outer periphery of the bundle.

In the light of observations made on earlier and slightly later stages, to determine the position of origin of protophloem (first sieve element), it is tentatively concluded that the first protophloem element initiates at about the middle region of a stamen, and then differentiates both acropetally and basipetally.

It is assumed that a similar sequence of stages occur for the differentiation of first elements of protophloem in the two lateral stamens.

By the time a stamen has the first protophloem[1] elements differentiated, the cells of the strand appear much denser and smaller in diameter than the adjacent parenchymatous cells in the region of the connective. This is most probably due to the higher vacuolation of the surrounding parenchymatous cells in this region. However, the cells of the strands are never found to be distinguishable from the surrounding cells in the filament.

Protoxylem:-

The differentiation of the first element of protoxylem occurs when two to four protophloem elements are more or less mature. It appears near the inner margin of the strand, that is the side towards the inner surface of the stamen.

By the time the first xylem element makes its appearance, the anther is an elongated structure borne on a fairly well developed filament (the filament is about 360 microns high).

Transections of young florets are used to determine the position of origin of the first xylem element.

The earliest stages in the differentiation of the first xylem element were observed in a stamen that measured about 936 microns in height. In such a stamen a patch of xylary elements extended for about 684 microns in length. Figures 12.6 to 12.9 illustrate the position of origin of the first protoxylem element in an anterior stamen. Figure 12.6 and Plate 28A depict the upper portion of the connective in

transections, with no indication of the presence of a xylary element. However, it can be easily distinguished from its neighbours, by its characteristic secondary thickenings, in a section about 60 microns below the top of the connective, which is represented in Fig. 12.7 and Plate 28B. It can still be detected in the young filament, Fig. 12.8, which is 252 microns further down, but no trace of it can be found below the point where the filament joins the floret axis, as may be noted in Fig. 12.9 and Plate 28C.

The above observations suggest that the first element of protoxylem initiates at about the middle region of the developing stamen, then later, elements differentiatesin both directions, i.e. its differentiation is also bipolar, like that of the first element of protophloem. These observations are in conformation with the opinion of almost all previous workers, with respect to the origin of first elements of protoxylem.

Before the first element of protoxylem differentiates, the procambial cells are comparatively small and their contents dense. As the stage of protoxylem differentiation approaches, the cells on the xylary side of the strand seem to enlarge slightly so that in transection they now appear larger and less dense than those on the phloem side. This difference in vacuolation of cells at the two poles of the strand is not as marked in the upper region of the connective as it is in the middle region.

Figure 12.

Position of initiation of protophloem and protoxylem in stamens.

Fig. 12.1 to 12.9, serial transections of young florets, illustrating the position of origin of the first protophloem element (12.1 to 12.5) and protoxylem (12.6 to 12.9), in the anterior stamen.

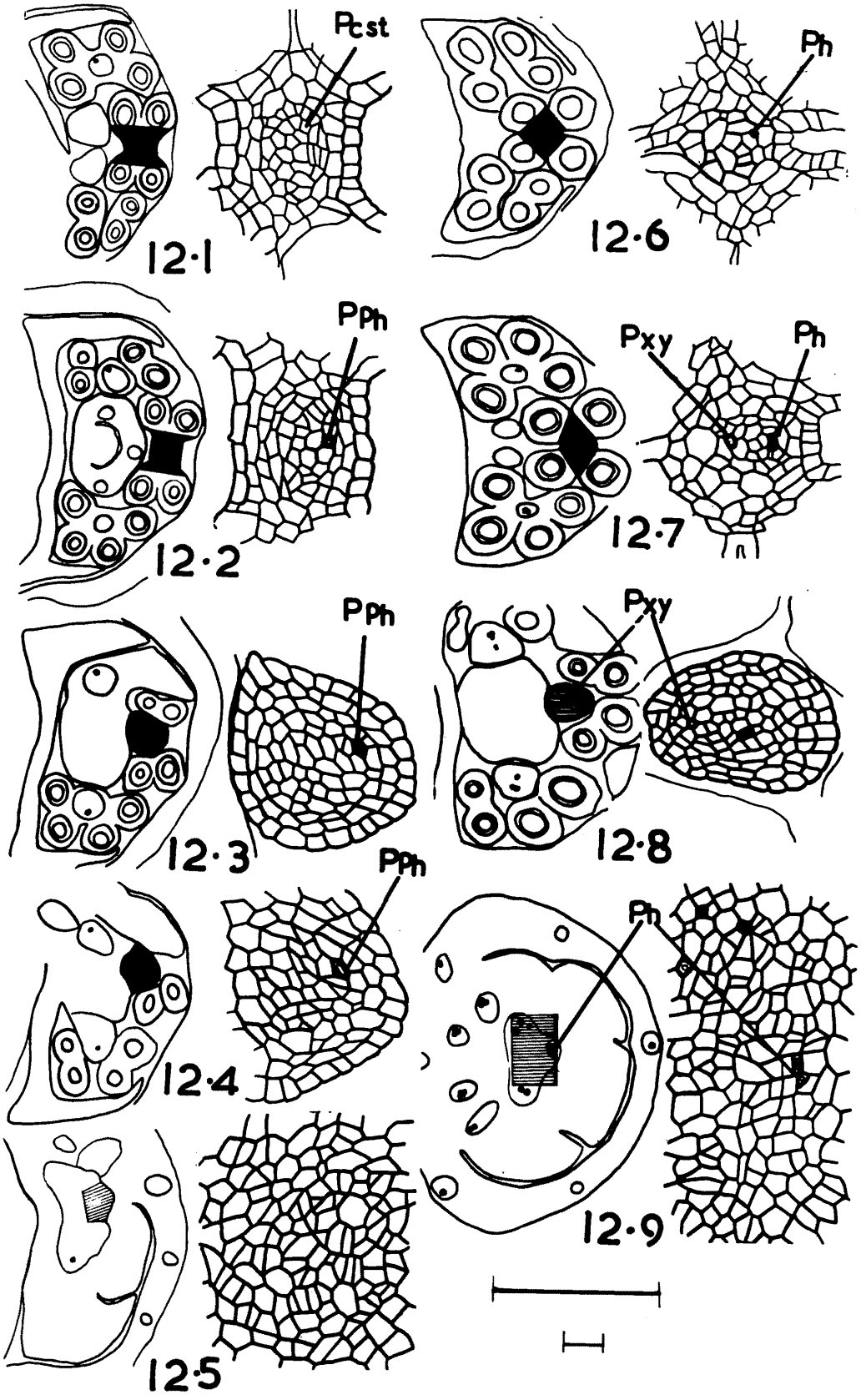

Pcst
12·1
Ph
12·6
Pph
12·2
Pxy
Ph
12·7
Pph
12·3
Pxy
12·8
Pph
12·4
Ph
12·9
12·5

Fig 12

Even after the differentiation of the first protophloem element, the procambial cells continue to divide, most of these divisions are periclinal with respect to the outer surface of the bundle. The resulting cells, when seen in transection, tend to arrange in radial rows between the group of differentiated protophloem elements and the first differentiating protoxylem element, though this orderly arrangement is soon disturbed.

Presumably the same sequence of events holds true for the initiation of the first protoxylem element in the two lateral stamens, although this was not investigated.

(b) Carpel.

(i) Course of strands in a mature carpel.*

The presence of four vascular strands is associated with the mature carpel of Tritioum, (one median, two laterals, and one funicular or placental strand). The median and two laterals are located in the peripheral region of the ovary wall, each opposite to the position of the respective stamen. Each lateral strand, after running up the ovary wall, enters a stigma, while the median, which is the smallest strand terminates in the ovary wall just below the top of the ovary. The funicular strand, which is the largest, lies opposite and runs upwards to end in the region of the attachment of the ovule.

* Here the word "Carpel" indicates the structure that is made up from a single foliar like structure and a part of the floret axis.

It was observed that even up to the stage of fertilization, the median and two lateral strands consist only of sieve elements. No indication of the presence of xylem elements was detected in them. Xylem elements may perhaps be produced after this stage, but this possibility was not persued.

However, the funicular bundle in its lower region, consists of both phloem and xylem elements as may be noted in Plate 30 D-F. It appears semicircular in shape when viewed in transection. The protophloem elements occupy the outer circumference of the circular group of cells and protoxylem elements are present in the centre.

In the case of both median and lateral strands, the elements of protophloem are surrounded by narrow elongated parenchymatous cells, giving the strand a circular shape in transection, though no proper sheath of cells is observed around it. The circular shape of the median strand is found to be less obvious than in the case of laterals.

The number of vascular elements is fewer in the median, more so in the lateral and most in funicular strand.

The mature elements of protophloem, in all the four strands, are characterised by having thick, deeply staining cell walls, unstainable cell contents (if any are present, as has recently been postulated by Esau (1963)) and enucleate condition.

(ii) Protophloem.

(a) Median and Lateral Strands.

In the early stages of origin of procambial strands, the procambial cells in all the three strands of the carpel appear slightly denser and show more avidity for stain uptake than the adjacent cells.

Later on the procambium cells become easier to distinguish partly because of relatively higher vacuolation of the surrounding cells and partly to their characteristic arrangement and position in the carpel wall.

The increase in thickness of the procambial strand prior to the differentiation of the first protophloem element is mostly by divisions of the cells on its periphery. As enlargement of the cells now follows each division, the diameter of the resultant procambial cells do not appear much less than that of the neighbouring cells in transections, though this tendency seems to be more marked at the inner pole of the strand (the xylem side) than the outer pole (the phloem side).

The earliest indications of differentiation of a phloem element are found to be the same as those described for the stamen. Until recently it was thought that the phloem element has recognisable and stainable cell contents up to the stage when the cell wall becomes thickened at the corners, but loses it at maturity. The work of Esau

(1963) suggests however, that a thin cytoplasmic layer is present even in a mature phloem element.

The examination of serial transections of young florets, suggests that in both the median and two lateral strands, the first element of the protophloem appears as an isolated entity, and is not in continuity with the phloem found further down in the more mature strands of the axis. Thus, it is not initiated as the result of a continuous upward propagation from below.

In all three procambial strands (median and two laterals) the first protophloem element can first be detected at about the middle region of the carpel. It appears earlier in the median than in the two lateral strands.

The first protophloem element is observed in the median strand of a carpel that measures about 1050 microns high and in the laterals by the time it is about 1410 microns high.

Figures 13.1 to 13.5 and Plate 29A - C illustrate the position of origin of the first protophloem element in the median procambial strand of the carpel. Figure 13.1 depicts the upper portion of the ovary wall in transection, with no indication of the presence of a phloem element, whereas it can be picked up very easily by its characteristic cell wall thickenings and by the absence of stainable cell content in Figs. 13.2, 13.3 and 13.4, but no trace of it could be found only 40 microns further down, as indicated in Fig. 13.5.

Figure 13.

Fig. 13.1 to 13.5, serial transections of a developing carpel, illustrating the position of origin of the first protophloem element in the median procambial strand.

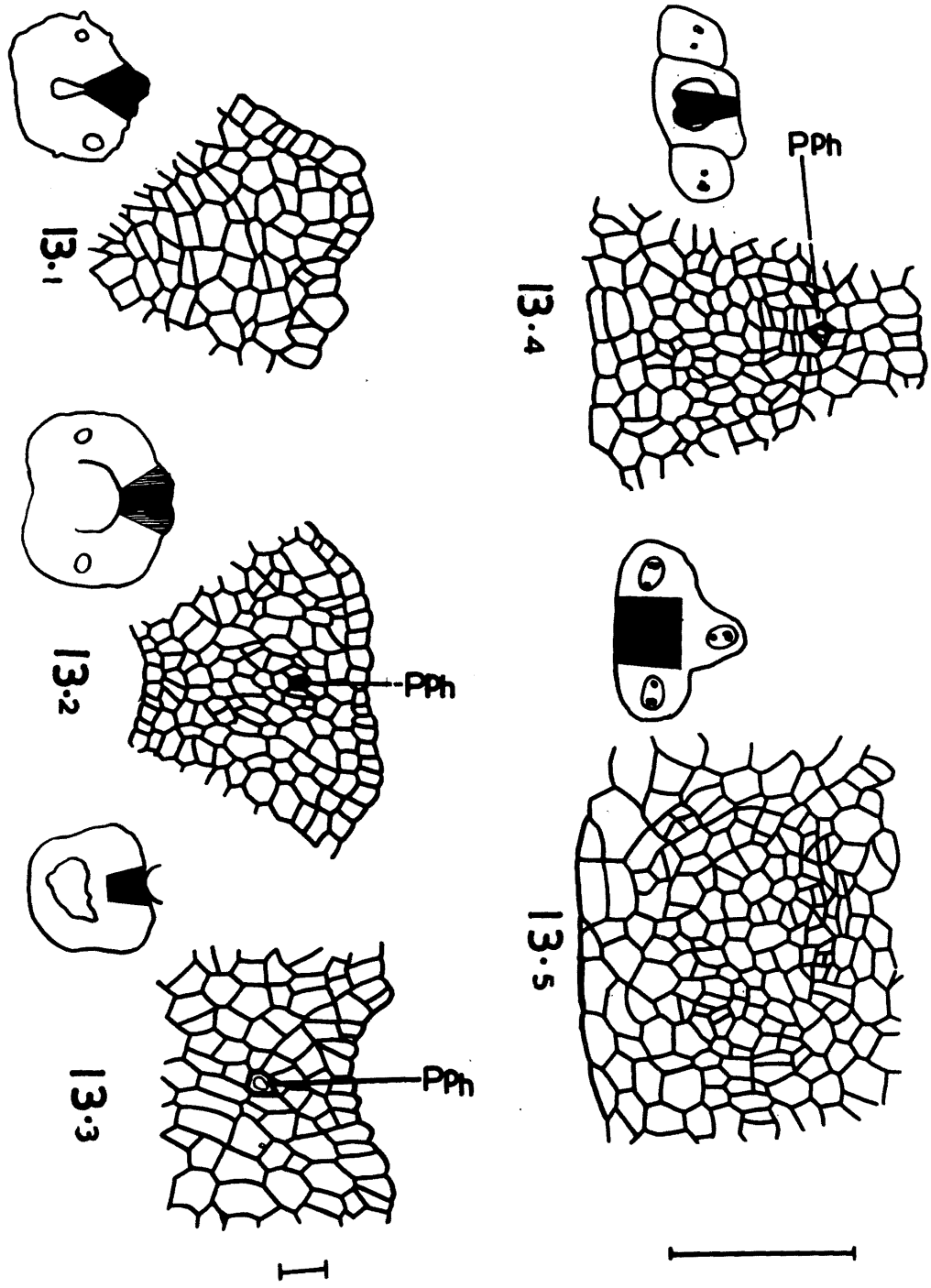


Fig13

In this particular material, protophloem was observed about 160 microns below the top of the ovary wall, and it extended for about 120 microns in length, and most probably was composed of three elements arranged end to end in a single row.

Plate 29B depicts an immature protophloem element. It may be noted that although its walls are already somewhat thickened at the corners and deeply stained (greyish black in the microscopic preparation), the cytoplasmic contents are still as dense and have stained similarly to the other procambial cells.

The examination of serial transections of more developed carpels show the presence of protophloem elements in both comparatively higher and lower regions of the strands. The above observations lend support to the conception of bipolar differentiation, that is, the first protophloem elements initiate in the central region, then they differentiate both acropetally in the carpel and basipetally to join the more mature strands in the axis.

(b) <u>The funicular strand</u>.

The funicular procambial strand also increases in thickness by the division of cells within the strand and by the addition of new cells on its periphery.

The appearance of protophloem and protoxylem elements in the funicular bundle is comparatively much later than in the median and the two lateral strands. The funicular strand is only represented by a patch of narrow elongated

parenchymatous cells in the lower portion of the carpel, when the other three strands are well differentiated, (see Figs. 11.1 and 11.2), and the ovule has fairly well developed integuments.

The examination of serial transections shows that the protophloem as well as protoxylem elements in the funicular strand develop in continuity with the phloem and xylem respectively, present further down in the more mature strands of the axis, and not as isolated entities, as observed in the cases of the median and two lateral strands. Plate 30A -F illustrates the differentiation of protophloem and protoxylem elements in the funicular strand, their absence in the upper portion of the strand (A-C) may be noted, while they are very well differentiated lower down (D-F).

Furthermore, despite careful examination of serial transverse sections of a number of mature carpels, in no case (in the funicular strand) could either protophloem or protoxylem elements be found, which were not in continuity with the same tissues in the axis below.

In the funicular bundle, the number of vascular elements decrease from the base upwards.

Comparative Anatomy.

AVENA.

The arrangement of three merismetic or germ layers (i.e. dermatogen, hypodermis and subhypodermis) in the spikelet apex and floret primordium is found to be similar to that of Triticum. Figures 14.1 and 14.2 are longisections of young apices of Avena spikelets, illustrating the organization of the above mentioned three layers in the spikelet apex as well as in floret primordium (Fp). The pattern of development of the lemma (L) and anterior stamen (As) is also depicted in the same illustrations, also as in Triticum the stamen of Avena initiates by the periclinal division of two to three subhypodermal cells, see Fig. 14.1. Whether these divisions are followed or accompanied by the periclinal division of hypodermal cells is not certain, however, the formation of new periclinal walls in these cells have been observed in later stages of development.

For the initiation of the lemma, one to two hypodermal cells undergo periclinal divisions Fig. 14.1, which are most probably followed, in the same number of dermatogen cells, by similar divisions. That most of the bulk of the lemma is derived from the dermatogen is evident from Figs. 14.1 and 14.2 at L.

The origin and course of development of the carpel in Avena is comparable to that of other members of the

Graminae. That is, the carpel initiates on the abaxial side of the floret axis by periclinal divisions of a few hypodermal cells, which are followed or accompanied by similar divisions of two or more dermatogen cells, see Fig. 14.3. It continues its initiation circumferentially around the axis by similar divisions Fig. 14.4 at C". The difference in the level of insertion of the ad- and abaxial sides of the carpel is not as marked as is observed in Triticum.

In the subsequent development of the carpel, the dermatogen contributes more than the hypodermis, as is evident from Fig. 14.6, where tiers of cells derived from the division of dermatogen cells for closing the top of the ovary can be seen.

The filament makes its appearance fairly late in the development of the stamen. Figure 14.5 indicates the commencement of development of the filament.

Plates 31 and 32 (A-C) are transections of a young stamen and carpel (respectively) illustrating the early stages in the differentiation of procambial strands in them. The illustrations shown in Plate 31 (A-C) are taken from sections, 48 and 24 microns apart and those depicted in Plate 32 (A-C) are 24 and 36 microns apart. In the material examined the procambial strands in both organs were found in continuity with the mature ones below, in the axis, although the outline of the procambial strand is more discernible in the middle region than below.

It indicates either that the differentiation of procambial strands in the floral parts of Avena is acropetal or that the material studied was older, where the procambial strands have already joined the mature strands of the axis after their independent origin.

Secale cereale (Rye)

The mode of origin of the carpel in Secale is similar to that found in Triticum. The pattern of dermatogen cell divisions in the early development of the carpel indicates, that this layer contributes most in the formation of its tissue. The difference in the levels of insertion of the anterior (median) and posterior portions of the carpel is even less than that observed in the cases of Avena and Triticum. The stages in the initiation and early development of the carpel are more or less comparable to those illustrated by Sharman (1945) for the vegetative leaves of Agropyron.

The early stages in the differentiation of procambial strands have been followed only in stamens, where they are found to be initiated as isolated small patches of elongated, darkly stained cells, more or less in the middle region of the organ. From here they differentiate in both directions, that is acropetally in the stamen itself and basipetally to join the more mature strands, down in the axis. In Fig. 14.10 owing to the fact that the section is not perfectly horizontal the two lateral stamens show the upper and lower extremities of their developing procambial strands,

where these are represented by a few periclinally dividing cells only, while the anterior stamen shows the middle of the developing procambial strand where it is relatively well developed. It should be noted that the outline of the procambium in the anterior stamen is more marked than in the upper lateral stamen (which is cut higher), and is wider in cross section than the one shown in the lower lateral stamen (which is cut lower than the anterior stamen).

In the illustration mentioned above, the section of the upper lateral stamen is 36 microns below the top of the stamen. The anterior and lower lateral stamens are sectioned 108 and 120 microns below the top of the stamen respectively.

In a section of the same florets 36 microns lower down, the procambial strand is not discernible in the lower lateral stamen, see Fig. 14.11, and the procambial strand in the anterior stamen is not clearly outlined as it appeared in the upper section (Fig. 14.10).

Hordeum distichon L.

Only a few longisections were taken to see the initial and developmental stages of the carpel, but the material was rather old for these stages and only the development of integuments could be observed.

In Hordeum also the ovule bears a pair of integuments (i.e. outer and inner). Each integument is composed of two layers of cells, the pattern of divisions in the

Figure 14.

Figs. 14.1 - 14.4. Longisections of developing spikelets of Avena.

Fig. 14.1. Shows the arrangement of dermatogen (D) and hypodermis (H) in the spikelet apex, and also the developing floret primordium (Fp), and lemma (L).

Fig. 14.2. Development of an anterior stamen (As).

Fig. 14.3. Initiation of carpel (C').

Fig. 14.4. Later stage in the initiation of the carpel (C' = the anterior and C" = the posterior portion of the carpel).

Fig. 14.5. Longisection of a young stamen of Avena (cut at right angle to the plane of glumes) showing beginning of the filament at its base.

Fig. 14.6. Longisection (at right angle to the plane of glumes) of a developing carpel of Avena, showing periclinal division in the dermatogen cells of "ovule", for the initiation of the integument and also repeated periclinal divisions of dermatogen cells on the inner face of the ovary, producing tiers of cells for closing the top of the ovary loculus.

Fig. 14.7. The development of integuments in Hordeum.

Figure 14 (continued).

Fig. 14.8 and 14.9. Initiation of the carpel in Secale.

Fig. 14.10 and 14. 11. Differentiation of procambial strands in the stamens of Secale.

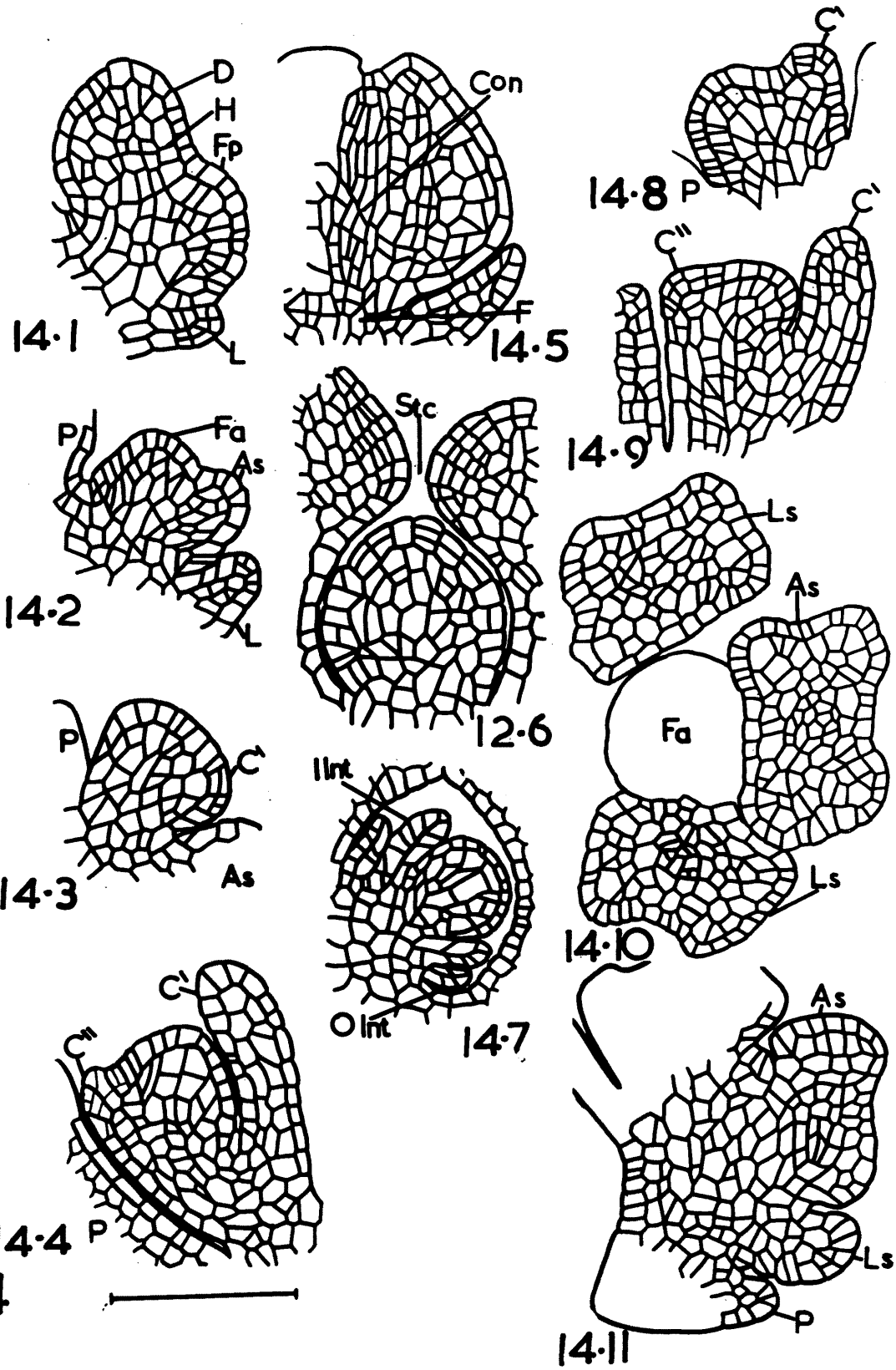

D
H
Fp
L
14·1
Con
F
14·5
C'
14·8 P
C''
C'
14·9
P
Fa
As
L
14·2
Stc
12·6
Ls
As
Fa
Ls
14·10
P
C'
As
14·3
IInt
OInt
14·7
C'
C''
14·4 P
As
Ls
P
14·11

Fig14

terminal cells indicates that growth is apical.

Figure 14.7 depicts the longisection of a carpel (in the plane of glumes) in which the upper and lower halves of both integuments (outer and inner) are seen to be equal. From this observation it can be assumed that in Hordeum the integuments initiate in the form of concentric rings, and the rate of growth is uniform all over the margin of these rings.

Oryza sativa (Rice)

Oryza sativa has been found most difficult, not only to orientate material, due to smallness of the florets, but also to preserve. Both F.A.A. and gluteraldehyde were tried. Although gluteraldehyde produced better results, it was still not satisfactory.

The course of initiation and early development of the carpel could not be followed due to the poor preservation of the tissues, however, the longisections of young florets, see Figs. 15.1 to 15.3 indicate that origin and development of the carpel in Oryza probably also follows the general pattern of initiation and development of the carpel in grasses.

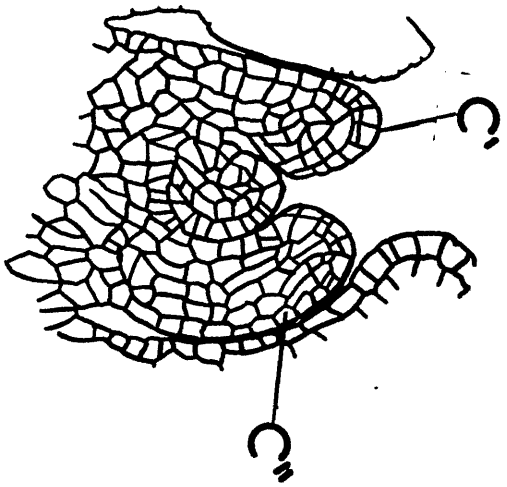
The early stages in the development of procambial strands were followed in stamens only. In the floret studied for this purpose there were only four stamens instead of the usual six. In all stamens, it was observed that procambial strands were in continuity with the mature strands of the

Figure 15.

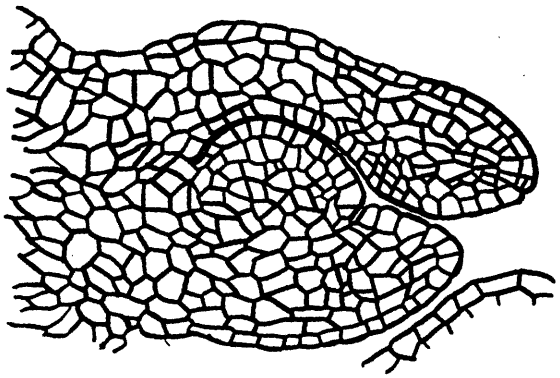
Figs. 15.1 to 15.3, longisections of florets, showing general pattern of development of the carpel at C' and C" in Oryza.

Note in 15.2, the tier of cells derived from the longitudinal divisions of dermatogen cells for closing the top of the ovary, and in 15.3 the periclinal divisions of dermatogen cells on the upper side of the future ovule, for the initiation of integuments.

15.1



15.2



15.3

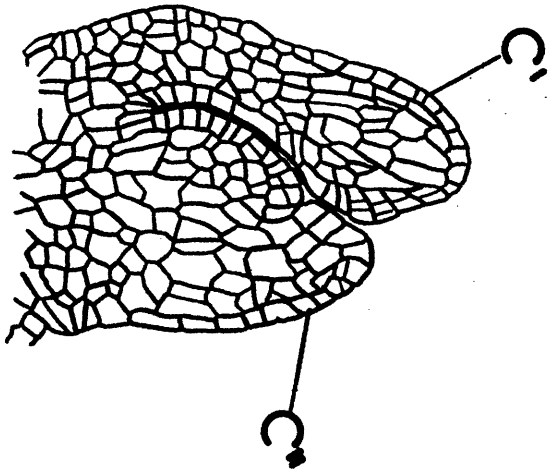


Fig15

axis, even if they had originally been initiated independently as isolated patches. To check whether the differentiation of procambial strands in floral appendages is actually in continuity with the older strands of the axis or whether the material studied was too old to show the earliest stages of procambial strands initiation, still awaits further investigations.

Figure 16.1 depicts the stamen in transection about 30 microns below its top, where the procambium is represented by a few dividing cells, whereas a detectable procambial strand is present in the illustrations shown in Fig. 16.2, and 16.3, which are drawn from the sections taken at intervals of 40 microns and 20 microns respectively, below the previous section. Figure 16.4 is the outline drawing to show the pattern of arrangement of palea and lemma, at the level shown in Fig. 16.3.

Figure 16.

Fig. 16.1 to 16.4. Differentiation of the procambial strand in the stamens of an Oryza floret.

16.1. T.S. of terminal portion of the stamen with no distinct procambial strand in it.

16.2. 40 microns below, a patch of longitudinally dividing cells is discernible in the same position.

16.3. 20 microns further below shows the presence of well differentiated procambial strands.

16.4. Outline drawing of the floral parts at the level shown in diagram 16.3.

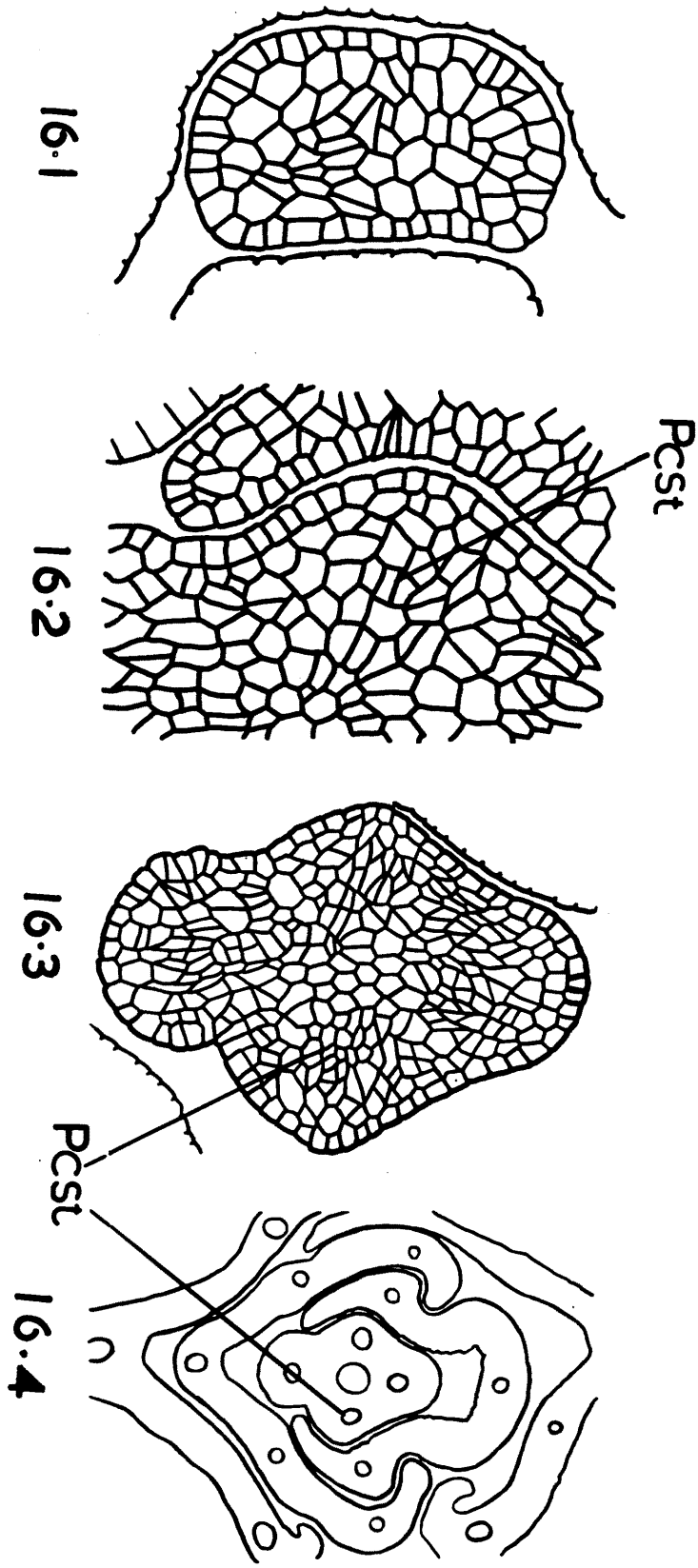


Fig16

Discussion.

The organization of three meristematic layers (i.e. dermatogen, hypodermis and subhypodermis) in a spikelet apex is similar to that of a vegetative apex, as described by Rösler (1930) for Triticum and by Sharman (1945) for Agropyron.

Periclinal divisions in the hypodermal cells, followed or accompanied by similar divisions in the dermatogen cells is found to be a constant feature for the initiation of all the leaf-like organs. A similar sequence of stages occur in the initiation of vegetative leaves, as observed by Barnard (1955) in Triticum, Bonnett (1961) in Avena and Sharman (1945) in Agropyron.

There is a striking similarity in the initiation and the early development of the lemma, palea, lodicules, carpel and the integuments.

It cannot yet be ascertained whether the main bulk of these organs is derived entirely from the dermatogen or from both the dermatogen and hypodermis. At any rate, the subhypodermis and the cells internal to this layer probably contribute nothing. In the cases of the integuments, it is found, that though periclinal divisions occur in the hypodermal cells, the resultant cells contribute nothing in their development, and the integuments arise by the sole activity of dermatogen cells. The present observations are in conformation with those of Barnard (1955) and Bonnett (196).

DISCUSSION.

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According to Holt (1954), in Phalaris and Dactylis, glumes, palea and lodicules are derived solely from the two layers of the tunica, (i.e. dermatogen and hypodermis), whereas the foliage leaves, sterile bracts and carpel are initiated in the tunica and derive a portion of their central mass from the peripheral layer of the corpus. However, his illustrations (Figs. 9, 10 and 11) do not indicate any difference in the initiation of foliage leaves, glumes, lemmas, sterile bracts and the carpel.

In the formation of floret and stamen primordia periclinal divisions are never observed in dermatogen cells. They are, however, always found in the subhypodermal layer, and do frequently occur in the hypodermal layer too.

In the material used for the present investigation, the floret primordium is initiated by periclinal divisions in the cells of the subhypodermal layer, which are regularly followed by similar divisions in the hypodermal cells. The hypodermal cell that lies towards the centre of the initial, enlarges before its division. Barnard (1954, 1957) holds that the division of the central cell is only occasional in T. aestivum and Bambusa, but almost always occurs in other species of Graminae examined by him.

Although the histogenesis of the floret primordium is not described by Holt (1954), while investigating the inflorescences of Phalaris and Dactylis, he included illustrations which show the periclinal divisions in the cells of

the subhypodermis and hypodermis in the expected positions, (Figs. 10, 11, and 12). Also, as pointed out by Barnard (1957) the limit of the "tunica" has been inaccurately shown by Holt.

The observations and most of the literature available on grasses have shown that the early ontogeny of the floret primordium is very similar to that of a spikelet primordium (Barnard (1954)) and an axillary vegetative bud (Hsü 1944, Bonnett 1961). Furthermore it is homologous to them (i) in having similar organization of its meristematic tissue, (ii) in bearing lateral organs, and (iii) in that, it is subtended by a "leaf-like" structure i.e. lemma. Thus it can safely be regarded as an equivalent structure to a leafy shoot though its axis is very short. A similar inference has been drawn by Barnard (1954) and Bonnett (1953).

The initiation of the stamen is essentially similar to that of the floret primordium. Barnard (1954) noted the periclinal divisions in the hypodermal cells on the lateral peripheries of the future floret primordium. Hypodermal divisions in the same position have been observed, in the case of the stamen, in the present investigation (Plate 8, A-C). Occasionally a hypodermal cell that lies in the centre of the initial divides periclinally, but does not enlarge as has been observed by Barnard (1954, 1957) in T. aestivum, as well as in other species of Gramineae.

Periclinal divisions of the cells of the subhypodermal layer are found to be characteristic of both the floret primordium and stamen initiation, whichare usually followed in the adjacent hypodermal cells by similar divisions. This mode of origin is comparable with that of an axillary vegetative bud, as illucidated by Bonnett for Avena and Hsü for Sinocalamus. In vegetative buds periclinal divisions may occasionally be found in the hypodermal cells, but not as a regular feature.

The similarity between the mode of initiation of a stamen, floret primordium and an axillary vegatative bud is very interesting. According to classical theory the stamen is a modified foliar structure, this conception has also been maintained by most of the relatively recent workers, who studied comparative morphology or histology of vegetative and floral apices in the dicotyledons (Brook 1940, McCoy 1940, Boke 1949, Tepfer 1953). Although their descriptions of the stages of stamen initiation do not differ from the present observations, at the same time, in connection with leaf formation, no periclinal divisions in the cells of the outer layer (dermatogen) were observed by them, and in almost all cases periclinal divisions in the cells of the outer layer of the corpus (subhypodermis) were encountered.

Presumably, periclinal divisions in the cells of the outer layer (dermatogen) in leaf formation in most of the

angiosperms (especially in dicotyledons) is rare. Thielke (1957a) stated, "such divisions in the T_1 layer and proliferation of the leaf epidermis itself by periclinal divisions appear to be more common among monocotyledons than among dicotyledons" (quoted by Clowes 1960). However the presence of the periclinal divisions in the outer layer of the corpus (at least in some cases) might be interpreted as of the similar nature to those observed by Barnard (1954) in relation to the origin of the lemma of T. aestivum.

Certian differences in origin have been enountered in the grasses, by almost all previous workers, between the initial stages of formation of a foliage leaf and of an axillary vegetative bud. Similar distinctions are found to be present between the origin of a leaf-like structure (lemma, carpel, etc.), and of a stamen, in the present study, which are also in agreement with Bonnet (1953, 1961), Barnard (1954) and Sharman (1960). Thus, it is logical to deduce that a stamen is bud like in its origin and it is an axillary cauline structure, at least in the grasses. Holt (1954), though he did not describe in detail the origin of the stamens with respect to the initiation of the inflorescense of both Phalaris and Dactylis concluded by saying, "Stamens and pistils are derived from both the tunica and the corpus". He has regarded stamens as "leaf-like" structures in their derivation, with respect to Avena (1955). However, his illustrations showing histogenesis of the organ are not

different from the ones presented in this investigation.

Tepfer (1953) comments that, "Recent workers in developmental anatomy have sometimes claimed it is possible to determine whether an organ is foliar or axial by examining the position of divisions in the shoot apex that initiate the organ".

He refuted the above claim by saying, "The question of whether a structure is axial or appendicular cannot be resolved by determining in which layer or layers of the shoot apex it originates (Foster 1949). Variations in initiating layers exist within the same species and between different species".

The present writer agrees with Tepfer, that up to a certain extent it is arbitrary to distinguish an organ from others, only on the basis of the seat of its origin, unless it is coupled with other aspects as well, in favour of this distinction (e.g. vascular anatomy, etc.), but at the same time it is reasonable to suppose that a division of a particular cell is not effected by the physiological activities of that cell only, but of those also encasing it. Thus, the regular occurrence of certian divisions, in certian regions, in a particular pattern,must carry some validity. As far as variations in the initiating layer or layers are concerned, the number of such instances encountered by workers is still limited and presumably they may be induced in nature, variations in the number of tunica layers (i.e. initiating

layers) have been induced by application of growth substances (indole-acetic and indole-propionic acids), by Ball (1944).

Despite this, the conclusion arrived at by Tepfer for the similar origin of all appendages, in the case of Aquilegia and Ranunculus, appears to be partly based on the grounds of their seat of origin.

The palea initiates and develops strictly as a foliar structure. Periclinal divisions in hypodermal cells, at its initiation, are a regular feature and not occasional as noticed by Barnard (1957) in other members of the Graminae. The initial stages for the palea and the stamens seem to be simultaneous, because in no sections (longisections) of floret primordia have the initial stages of one been observed without those of the other. Barnard (1954) thinks the palea begins its initiation earlier than the stamens. However, the illustrations given by him do not support this view (Figs. 6D, and 7A, Plate 4, and Figs. 3 - 6).

Very shortly after the initiation of the palea and the stamens, genesis of the lodicules occurs. Barnard holds that the stamens and the lodicules initiate at the same time, although his illustrations do not indicate that this is so (Plate 4, Fig. 4).

Holt (1954) found that the lodicules are foliar in nature; according to him each lodicule derives exclusively from the "tunica" (i.e. dermatogen and hypodermis).

In the present study their initiation and early development has been found to be similar to that of leaves or leaf-like structures, that is, in complete agreement with Barnard (1954). Arber's studies on their morphology, also lend support to the view that they are foliar in nature. Furthermore the pattern of their vascularization also strengthens the present contention.

Bonnett (1953, 1961) regarded lodicules as axial structures, according to him they initiate and develop due to the activity of the hypodermis and subhypodermis, a characteristic ascribed to the origin of a bud or bud-like structure.

It seems most likely that Bonnett observed the area that is situated just below the anterior stamen (or immediately on either side of it), where lodicules are only being initiated by periclinal divisions of hypodermal cells, as the periclinal division in the cells of the dermatogen layer and further development of the two lodicules is restricted to the two areas occurring between the anterior and two laterally placed stamen primordia.

The periclinal divisions in the cells of the subhypodermal layer, at the time of initiation of lodicules, has also been observed in the present study. The nature of these divisions might be similar to those occurring in relation to the origin of the lemma, as observed by Barnard (1954) in Triticum. He states "A periclinal division of several sub-

hypodermal cells may occur. These cells do not divide again for some time so that the subhypodermis contributes nothing to the tissue of the lemma". This aspect has not been persued closely (for the lodicules) in the present study, though by the pattern of the arrangement of cells in their early developmental stages, it can be presumed that only the outer two layers (dermatogen and hypodermis) contribute to their tissue (see Plate 10C, 11C, 12B).

These periclinal divisions in the cells of the sub-hypodermal layer may be concerned with the increase in circumference of the floret primordium for, as may be noted in Plate 10 A-C, these divisions of subhypodermal cells are not restricted to the initiating region (vertical plane) of the lodicules, but extend over the whole area of the floret primordium.

The pattern of the vascular system observed in each lodicule is in conformity with the views of Arber (1934) and Bonnett (1961), though the vascular strands in the body of the lodicule of the material studied, consist of more vascular elements than described by Bonnett in Avena.

Each vascular strand (at least in the lower regions) consists of both xylem and phloem elements. The absence of the phloem elements have been reported by Zuderell (1909) for about 50 species of Graminae (quoted by Arber), and by Knoblock (1944) in Bromus, but it is felt that for Bromus, at least, this needs re-examination.

The nature of the carpel in grasses is still the subject of controversy, for this reason particular attention has been given to its early ontogeny in the present study.

The initiation and early developmental stages of the carpel are very ~~much~~ comparable to the origin of a typical grass leaf, that is, it is initiated on the anterior side of the floret apex by periclinal divisions in the cells of the hypodermal layer, followed by similar divisions in the dermatogen cells. From here the divisions spread laterally around the base of the floret apex. Before completion of this lateral growth the protrusion formed by the earlier divisions begins to grow upward. Thus apical growth and lateral expansion combine to produce a structure that is highest at the point of its origin and slopes down along the margin.

This account of the initiation of the carpel is in complete agreement with those of Barnard (1954, 1957) for T. aestivum, Bonnett (1953, 1961) for Zea and Avena and Sharman (1960) for Anthoxanthum.

Holt (1954) holds that both in Phalaris and Dactylis the carpel derives from both tunica and corpus. However in the illustration (Fig. 12, P.617) given by him to support this conclusion, the limit of the tunica has been shown inaccurately.

The grass carpel (or gynoecium of other workers) initiates and develops as a single entity, throughout its genesis. It does not give the slightest indication of being a syncarpous

gynoecium. If the usual concept of a tricarpellary gynoecium is accepted (that is generally postulated for the gramineous gynoecium) then three carpels should be represented at least in the early ontogeny of the carpel.

Contrary to this, after it originates on the abaxial side of the floret axis, the initiation of the gynoecium is continued round the axis, and it develops as a single lateral structure.

The same sequence of events in the ontogeny of the carpel has been observed by Barnard (1954) and Bonnett (1953). Bonnett was not, himself, sure about the tricarpellate nature of the Zea carpel so he commented, "The tricarpellate nature of the pistil is not indicated from its external appearance during its initiation. It appears to be a unit.---. The tricarpellate nature of the maize pistil must be arrived at from evidence other than obtained from a study of its initiation and early stages of development". Esau (1953) also thinks that the grass gynoecium arises as a single unit, and does not reveal ontogenetically the three carpellate structure generally associated with the Gramineae flower.

With respect to Phalaris and Dactylis, Holt (1954), states "The ontogeny of the carpel, in the grasses of the present study, does not show tricarpellate condition. On the contrary, the carpel develops as a single foliar structure and is uniloculate at maturity".

On the basis of study of vascularization of adult florets only, Arber (1934) has interpreted the grass gynoecium as tricarpellary in nature. According to her, the median and two lateral traces represent the midribs of three carpels, while the funicular bundle (placental)and two more bundles (x - y)* which occasionally occur are formed by the fusion of marginal bundles of these three carpels. This view was supported by Chandra (1962) on similar grounds. On the first hand, the validity of this interpretation is doubtful as it is based on the study of vascular system of adult carpels. Even if it is accepted it is difficult to see what explanations could be advanced for the following:-

(a) Why the median and two lateral strands represent the mid viens of three carpels, when with equal justification they can be regarded as three strands of a single carpel.

(b) What conclusions should be drawn for those instances where the number of vascular strands in a carpel is less than three, e.g. in Nardus stricta, where the single style is served by one anterior strand only and one posterior (funicular) strand supplies the ovule.

*Foot Note:- Arber has regarded (in some bamboos), the median strand as "c" two laterals as "b" and "a" and the two strands that occur occasionally between the median and two laterals as "x" - "y".

> If, according to Arber's interpretation in the above example (b), the midribs of two lateral carpels are missing, or in other words two lateral carpels have been reduced to such a point as to lose not only their external individuality but even their vascular strands, then how can the presence of the presumed fused marginal strand (funicular) be explained?

Again, in cases such as Danthonia, where the carpel has no median strand (quoted by Barnard 1957) a tricarpellate interpretation is hard to accept.

In the present investigations, the findings on the vascular strands of the carpel indicate that the funicular strand is not only different from the other three bundles (median and two laterals) in occupying a bigger area in cross sections (in the lower regions), but also differs in its early ontogeny and adult structure.

(1) The procambia originate as isolated patches of tissue in the positions of the future median and lateral strands, then their further differentiation is bipolar. The same holds true for the origin and differentiation of the first element of protophloem in the median and lateral strands, whereas the differentiation of the procambium as well as the vascular elements in the funicular strand is acropetal.

(2) Only sieve elements have been found in median and lateral strands (up to the time of anthesis) while very distinct xylary elements have been observed in the funicular strand.

(3) The funicular strand is more or less semi-circular in its shape, on the other hand Arber's (1934) outline drawings of x and y strands (whenever they are present) of certian Bamboos suggest, that these strands are more similar in their outline to median and laterals than to a funicular strand.

(4) Furthermore, after examining serial transections of fairly well developed carpels, it appeared that the funicular strand does not represent a large single bundle; it appears more like a group of small strands.

In the light of the above observations, one is tempted to conclude, that the posterior wall of the grass gynoecium with its bundle is different from the anterior one and is most probably axial in nature, representing the part of the floret axis just below the terminus, which bends downwards and develops into a single ovule (Barnard 1954, Bonnett 1953, 1961).

The median and lateral strands represent the strands of a single carpel, the number of these strands may vary occasionally.

This concept is also strengthened by observations on the order of differentiation of procambial strands in

Triticum leaves, where it is found that three procambial strands ("median" and two "laterals") appear first, followed by others, as may be noted in Plate 25 at post. The median bundle is reduced perhaps due to the pattern of growth of the carpel in Triticum. Arber observed its extension into the anterior "style" (whenever it is present), in some of the bamboos (e.g. Schizostachyum).

The foliage leaves of Crocus, have an interesting similarity to Triticum carpels, in that each has a very small median bundle in comparison to laterals.

It appears that there is a divergence of opinion about the position of insertion of three carpels on the floret axis. Bonnett (1953) thinks that the side of the floret apex next to the lemma bears two carpels and that one is born on its adaxial side i.e. next to the palea, whereas Arber's description suggests that one is anterior and two are postero-laterals in position.

Barnard (1957) holds that the grass gynoecium is composed of "three, and possibly four fused foliar structures".

The view of the presence of four carpels in a grass gynoecium (at least in Triticum) is not supported histogenetically, even in the light of Barnard's own observations (1954, 1957), which are in complete agreement with the findings of the present investigation.

He came to the above conclusion because he recognised an anterior, two lateral and a posterior unit to the grass

gynoecium, and postulated, they do not represent the parts of a single carpel, but that each constitutes an individual carpel, as they are inserted at different levels on the floret axis.

Another interpretation of the sloping insertion of the carpel seems to be explained as follows:-

(i) The insertion of the grass foliar leaf is also sloping because it is initiated first on one side of the shoot apex, then continues its initiation gradually at a higher level circumferentially around the axis (Sharman (1945)), although the difference in the insertion of the two sides of leaf is not so marked as in the carpel. In the carpel, this may be due to bending of the apex, produced by elongation of the cells in the posterior region of the apex, which might be pushing the region of initiation of the carpel upwards.

(ii) A sloping insertion of a single carpel might be the result of some physiological factor, so that the cells far from the apex (on the posterior side) are less metabolically active than the cells nearer. Whether or not there is such a difference in metabolic activity, and if so, what are its causes, must await further investigations.

The illustrations depicting the initial stages of the carpel of Secale (Figs. 14.8 and 14.9) do not show such a

marked difference in the level of insertion of anterior and posterior portions, as is noticed in the case of Triticum and Avena. It is also noted that the bending of the developing ovule is not as marked as in others (Triticum, Avena) in the early ontogeny of the carpel.

Probably the angle at which a developing ovule bends in the early ontogeny of the carpel and the angle of the insertion of a developing carpel are inter-related.

In support to the individuality of lateral carpels, Barnard, putting forward another argument, says "Towards the tip of the style, in the stigmatic region, there often appear in addition to the main provascular strand one or two other small groups of provascular like cells. One of the groups is situated at each side of the styler branch near its inner surface and the general structure of each branch of the style at this level is comparable to that of the tip of a young lemma".

In the material used for the present study, the presence of such provascular like cells near the inner surface of a stigma (styler branch) could not be detected. Perhaps Barnard has mistaken for provascular strands, the tissue which Essu termed as "stigmoid tissue". Furthermore the illustrations given by him, for the comparison between the tips of lemma (Plate 2, Fig. 4) and styler branch (Plate 2, Fig. 2) are not comparable in the true sense.

One of the reasons Barnard advanced for postulating the posterior wall of the carpel as an individual unit is:- "The occurrence of a leaf gap in the provascular cylinder of the axis in that position which a trace to a posterior unit would submit". Careful examination of serial transections of the developing carpels left no doubt that this "gap" (posterior) is non-existent in the material studied.

Barnard referred to Arber (1929), concerning Bambusa nana Roxb. and species of Ochlandra, where she has described the occurrence of six vascular traces and five, six or occasionally more styler branches in the gynoecium and concluded, "that more than four carpellary units were involved in the gynoecia of the prototypes of the gramineous flower".

What explanation would Barnard advance for those foliar structures, which bear more than one projection at their margins and their singular entity is an accepted factor? For instance, in Aegilops ovata L. both glumes (see Plate 6D) and lemmas are provided with a number of projections, each being supplied with a vascular strand. In Melica uniflora, Retz a curious projection is noticed, that develops from the top of the "fused" edges of the leaf sheath. This projection is a slender bristle like structure, 1 to 4 mm. long, which occurs on the side opposite the leaf blade, as may be noted in Plate 6C, yet the single nature of the leaf in Melica has not been doubted on this ground.

It is also very interesting to note, that a temporary

phase, that occurs in the development of the flag leaf of Secale is comparable to that of the Triticum carpel. In Secale, for a short time the growth is more active at two regions along the lateral margins than at the tip of the leaf. As a result two small protrusions are formed at the lateral margins, as may be noted in Plate 6A and B. However, soon after, the active growth of the tip is renewed and it assumes the typical shape of the foliage leaf.

The ovule appears to be terminal in position throughout its development, there are no evidences in ontogenic development of histogenesis to show that the ovule in Triticum is axillary as postulated by Barnard (1957).

Both integuments commence their initiation from one and the same side of the ovule (upper side), i .e. they do not alternate in their position of origin on the ovule as was expected. The inner integument is initiated earlier earlier than the outer one.

Histogenesis of the carpel, therefore, strongly suggests that the carpel in grasses (at least in Triticum) is a single "leaf-like structure". It originates on the anterior (or abaxial) side of the floret axis, then continues its insertion around the floret axis at gradually higher levels. As a result, the posterior wall of the carpel, for most of its length, is axial in its nature, as indicated (diagrammatically) in Fig. 17.4. The top of the posterior region of the carpel is formed by the fusion of the two margins of the carpel.

Figure 17.

Figs. 17.1 to 17.3, diagrammatic representation of the origin and direction of propagation of three procambial strands (median and laterals) in the carpel. The pattern of origin and differentiation of the first phloem element is similar. (These diagrams have been constructed after examining serial transections of carpels of different ages).

Fig. 17.4, diagram to interpret the insertion of the carpel on the floret axis (the lateral and the lower margins of the carpel are shown in dotted lines).

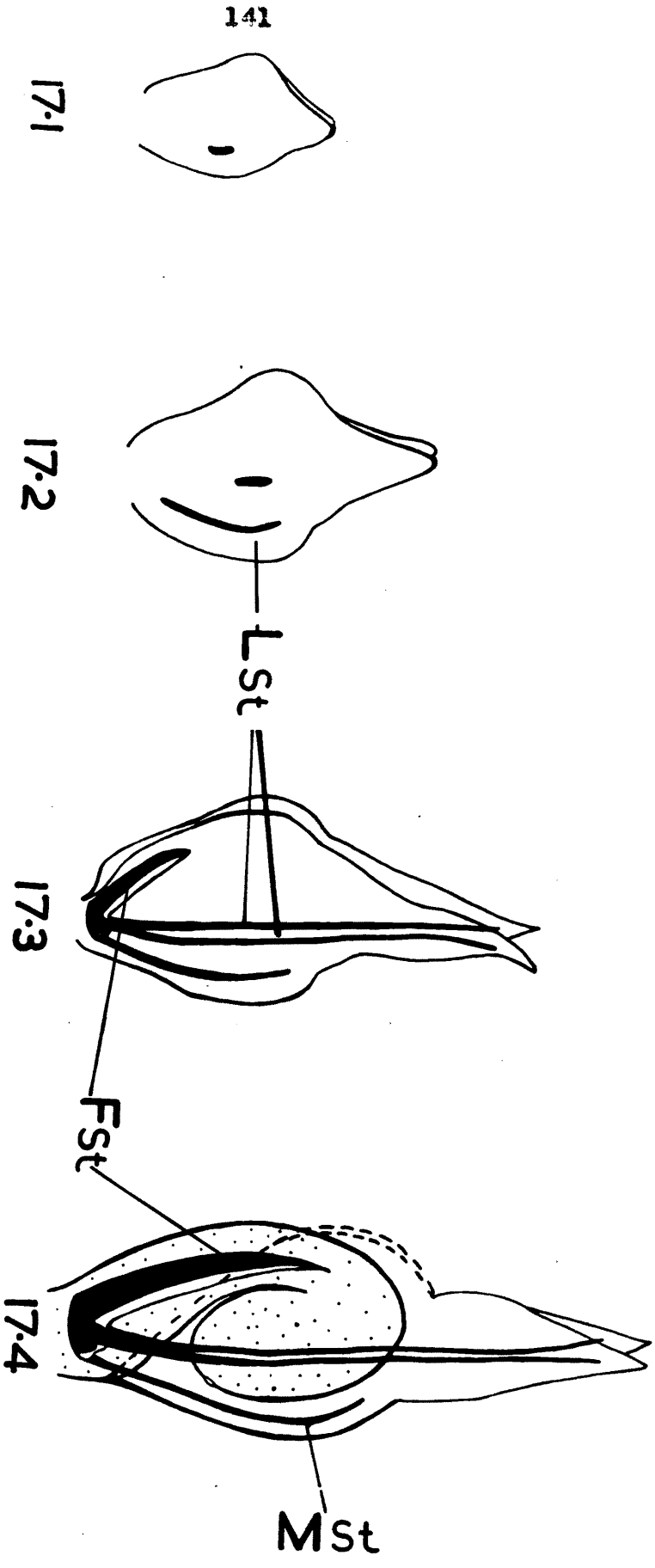

Fig17

Most probably, the anterior half of the vascular cylinder, (which is present at the base of the carpel), is formed by the "descending" vascular elements of the median and two lateral strands; whereas the vascular elements of the posterior region are acropetal in their differentiation and are continued in the funicle of the ovule as the "funicular strand", as may be noted in Fig. 17.4.

In the light of observations made in the present investigation, it is concluded, with respect to the procambium, that each procambial strand originates as an isolated patch of cells (a) in the position of the future median and lateral strands of the carpel and (b) also in all three stamens, at about the middle region, by the longitudinal divisions (parallel to the long axis of that organ) of a few cells. Then it continues its initiation both acropetally and basipetally by similar divisions.

No detailed account has yet been found about the origin and differentiation of procambial strands in monocotyledon flowers (at least in grasses). Bonnett (1961) seems to be the only one mentioning differentiation of procambial strands in the floral parts of grasses; he thinks that procambial strands develop acropetally in styles. His conclusion is open to doubt, as he studied only rather old material. However, acropetal, bipolar or even entirely basipetal differentiation have been reported for monocotyledon leaves.

Hsü (1944) holds that the differentiation of procambial strands in the cataphylls of Sinocalamus is bipolar. He states, "Provascular cells originate at the base of cataphyll, then differentiate both acropetally into the foliar primordium and basipetally to become connected with older strands below".

Sharman (1942) suggested all three types of differentiation amongst the procambial strands of the various types of bundles found in Zea leaves.

According to him, the median procambial strand of the leaf differentiates acropetally from the axis into the leaf (he also assumes that it has a basipetal course in its lower part within the stem), the laterals differentiate basipetally into the axis and acropetally into the leaf from somewhere near the base of the leaf, whereas smaller strands (strands interpolating between the lateral ones) differentiate basipetally and appear at the apex after the laterals reach their highest positions.

Bugnon (1924) has also noted that the initiation of a procambial strand in Melica and Dactylis leaves occurs independently of all the pre-existing conducting system, lower down in the stem. Further differentiation of this procambial strand is bipolar, that is, it differentiates acropetally in the leaf to form the median bundle and at the same time pursues very rapidly its differentiation towards the base of the stem.

Clowes (1960) supports Sharman's view for the longitudinal order of differentiation of the procambial strands in Zea mays leaves by saying "It may, at first seem odd that the vascular system of a leaf should differentiate partly acropetally and partly basipetally. But the state of differentiation depends on the age of the cells and this depends on the positions of the meristems. Until the primordium is three plastochrons old in Zea, meristematic activity is greatest at the tip, therefore, the basal cells, in general, have spent a longer time since the last mitosis than have the cells at the tip. During this period we might expect acropetal differentiation and this is what is found. When the primordium is older, the meristem is at the base of the leaf, the cells at the tip are therefore the oldest, and differentiation is basipetal".

Clowes further states that "Many monocotyledons have a meristem at the base of the leaves which persists beyond the primordial stage..... and it may be that it is a result of the presence of this intercalary meristem that some plants do show a reversal of the normal direction of differentiation in the procambial strands". If this is so, it might be presumed, that the presence of such intercalary meristems in the developing stamens and carpel of Triticum is responsible for this bipolar differentiation of the procambial strands.

Esau (1944) also seems to co-relate the direction of

differentiation of procambial strands with the maturation of cells in that particular organ, as she says, while commenting on the basipetal differentiation of smaller strands in Zea mays leaves, "the order of the appearance of the smallest bundles is related to the basipetal maturation of the leaves - a characteristic very common among angiosperms".

The recent work of Sharman and Hitch (unpublished) on the origin and direction of differentiation of procambial strands in the leaves of Triticum also lends support to the bipolar differentiation of the procambial strands in the stamens and carpel of Triticum. Their observations are in complete conformation with the present findings.

For most instances of dicotyledons, the acropetal differentiation of the procambium in the floral appendages is reported by recent workers (Boke, 1948, 1949, Miller and Wetmore 1946, and Tepfer 1953). But, in few cases, a bipolar course of differentiation of procambial strands has been recorded. Lawalrée (1948), referring to certian Compositae, holds that the procambium of the corolla, the stamens, and the carpels arises in isolated loci near these structures and then differentiates in two directions. Arnal (1945) reported a similar course of procambial differentiation for petals, stamen, and carpels of certian Veilaceae.

In the present study, the similar course of bipolar differentiation has been recorded for the initation and differentiation of the first element of protophloem in the

median and two lateral strands of the carpel and in the single strand of all the three stamens. It holds also for the origin and direction of differentiation of the first element of protoxylem in the stamens.

No substantial information is yet available on the origin and direction of differentiation of first vascular elements in grass florets.

Bonnett (1961), presumes that the differentiation of the vascular bundles is acropetal in grasses, as he has mentioned concerning the carpel of Avena, although his observations seem to be based on the structure of the mature carpel.

Barnard (1957) and Esau (1943) ascribed the discontinuity of the protophloem elements to the presence of an immature sieve element between the mature ones.

In the present investigation, the first element of protophloem is found to be discontinuous from the more mature phloem elements of the axis, both in the stamen and carpel. This interruption has been observed regularly at a certian level, at a definite stage of development of that particular organ.

If this gap is due to the presence of an immature sieve element, the position of this interruption might be expected to vary. Furthermore, an interruption of this kind would produce more gaps, which presumably might occur anywhere along the length of the young strands. In addition, a

mature sieve element is never found lower down, in the same relative position, at this stage of development.

The present observations on the origin and propagation of first protoxylem elements are in complete agreement with those of most of the workers (Trécul, Scott and Priestley).

SUMMARY.

The arrangement of three layers (dermatogen, hypodermis and sub-hypodermis) in the floret primordium, and spikelet apex is similar to that of vegetative apices. The similarity in the initiation of lemma, palea, lodicules, carpel and integuments is striking. The periclinal division of a few hypodermal cells followed or accompanied in the dermatogen cells by similar division, is found to be a constant feature for the initiation of these leaf-like structures.

On the contrary, periclinal division in the dermatogen cells is never observed in connection with the initiation of the floret primordium and stamens. Instead these arise by periclinal division in the cells of the sub-hypodermis, followed by similar division in the hypodermal cells.

The integuments arise almost entirely from the repeated division of dermatogen cells.

Each stigmatic hair develops by elongation and characteristic division of a single epidermal cell of the stigma.

A main vascular bundle enters the base of a lodicule. Each bundle in turn divides into three branches which by repeated divisions ramify throughout the lodicule.

The origin and direction of differentiation of the procambial strands was followed both in the carpel and stamen and it has been found that they appear as isolated patches of cells more or less in the middle region of the organ, then differentiate both acropetally and basipetally. A

similar pattern of origin and differentiation has been observed for the initiation and propagation of the first elements of protophloem in all three strands of the carpel (medium and laterals) and in the single strand of the stamen. It holds also for the first element of protoxylem in the stamen. The differentiation of the procambial strand and the vascular elements in the funicular bundle is acropetal.

The comparable stages for the initiation of the carpel and the differentiation of the procambial strands have also been observed in Avena, Oryza and Secale.

APPENDIX

APPENDIX I.

Some electron microscopic observations on the fine structure of a young differentiating sieve element.

(a) INTRODUCTION:-

Investigators of the ultrastructure of the protoplast of higher plants have mostly given their attention to the meristematic or other relatively undifferentiated cells; though there have been a considerable number of reports on phloem structure. Both light and electron microscopic studies have shown the presence of a peripheral cytoplasmic layer in a differentiated sieve element. This layer is semipermeable and permits plasmolysis (Currier, Esau and Cheadle 1955). It contains a considerable development of the endoplasmic reticulum, and in addition mitochondria, plastids and other bodies.

It seems now well established that the cytoplasm of sieve elements is unique in retaining many characters of living cytoplasm whilst losing others. Thus the nucleus disappears on maturity and the evidence seems to indicate that the tonoplast does too.

In order to appreciate the functioning of sieve elements, it is of prime importance to understand their fine structure.

(b) MATERIAL AND METHODS.

For electron microscopy the young stamens of a spring variety (Svenno) of Triticum aestivum were used. The young florets were dissected under a binocular microscope in a moist atmosphere, at regular intervals during maturation. The stamens having a length of about 600 microns were selected for study.

This length was chosen after examining serial cross sections of young stamens under the light microscope and ascertaining when the first protophloem element was well differentiated.

Suitable single stamens were transferred to a microscope slide and covered with a drop of selected electron microscope fixative. Each stamen was then immediately sliced transversely into two pieces to assist penetration and at once carried to a small tube containing fresh fixative with the help of a wide mouthed pipette.

1.5% gluteraldehyde in phosphate buffer of strength 0.01M (pH 7.0) was found most satisfactory as a fixative. It was allowed to act for four to twelve hours (over-night). Post fixation was carried out in 1 - 2% OsO_4 in the same buffer, for one to two hours.

The material was dehydrated through an alcohol series (ethanol) followed by a propylene oxide series. It was then embedded in epoxy resin epon (details in appendix).

(c) OBSERVATIONS.

Due to unavoidable circumstances it was only possible to do a little work on the first differentiating protophloem elements of the stamen. The following remarks may however be made on the electron micrographs presented (Plates 33, 34, 35 and 36).

There was unfortunately little opportunity for perfecting the specialised techniques of fixing, embedding and cutting, therefore, the sections are too thick to show the finest details.

Plates 33 and 34 show an early stage in the development of a sieve element. The wall has already acquired the characteristic thickenings, but it is difficult to positively identify the cytoplasmic inclusions. The large central dark area is probably the nucleus.

In conformity with this supposition, instances from light microscopy can be cited. Plate 28B at Pph certainly seems to suggest that the differentiating protophloem elements has not lost its nucleus yet. It is found after examining the serial sections, that the darkly stained structure occupies the longitudinally central region of the elements only and the ends of the latter appear empty. Plate 33 illustrates two sieve elements in transections, probably one with a nucleus represending the central region of the element, the other having been cut nearer an end and so appearing empty.

Bouck and Cronshaw (1965) reported, that during the later stages in the sieve tube development the contents of the nucleus disintegrate and ultimately the chromatin and nucleolus can no longer be recognised within the nucleus. However the nucleus is still bounded by its usual envelope. This may be the state of affairs in the present case.

Plates 35 and 36 may illustrate later stages, and seem fairly typical of mature sieve tubes (but contra Plates 33 and 34). The larger inclusions are probably plastids and the smaller mitochondria. The fairly common endoplasmic reticulum running parallel to the thick nacreous wall (and most likely sending processes perpendicularly into plasmodesmeta traversing the wall, see arrow) appears to be in evidence.

The tonoplast has apparently disappeared as usual.

Bouck and Cronshaw (1965) have shown the presence of plastids, mitochondria and endoplasmic reticulum in the differentiating sieve elements of Pisum, and this indeed has been the fairly common observations. According to them, mitochondria characteristically undergo a reduction in size in the early sequence of differentiation, but otherwise appear structurally similar to mitochondria elsewhere in the stem. They have referred to the endoplasmic reticulum in the differentiating sieve element as sieve tube reticulum.

Note:- The black spots are probably extraneous contamination.

APPENDIX II.

(a) Details of the methods used for light microscopy.

Formulae of fixatives.

(i) Acetic acid (a) Glacial acetic acid 5 c.c.

(b) $ZnCl_2$ 5 gms.

(c) formalin (40% soln.) 10 c.c.

(d) Ethyl alcohol (70%) up to 100 c.c.

(ii) Citric acid (a) Citric acid 0.5 gm.

(b) formalin (40% soln.) 10 c.c.

(c) water up to 100 c.c.

Staining. I. Tannic acid and Iron alum with Safranin and orange G.

Slides were dipped in xylene after being warmed in a flame to melt the wax, then brought down to water by passing through a gradual alcohol series. After rinsing with water they were transferred to:-

(1) Aqueous $ZnCl_2$(2%) for one minute.

(2) Rinsed with water.

(3) Five minutes in aqueous safranin ($^2/25,000$).

(4) One minute in orange G (see below).

(5) Rinsed in H_2O.

(6) Five minutes in tannic acid (see below).

(7) Rinsed with H_2O thoroughly (at least two changes of H_2O).

(8) Two minutes in aqueous Iron alum (1%).

After rinsing with water the sections were dehydrated by passing the slides through a gradual series of alcohol

(i.e. 45%, 70%, 90% and absolute alcohol) and then to xylene, mounted in Canada Balsm.

Orange G solution prepared from:-

Orange G = 2 gms.

Tannic acid = 5 gms.

Salicylic acid = few crystals.

Few drops of concentrated HcL.

Water = upto 100 c.c.

Tannic acid:-

Tannic acid = 5 gms.

Salicylic acid = Few crystals.

Water = upto 100 c.c.

II. Methyl green and pyronin stain.

(1) After removing the paraffin, slides were taken down to water.

(2) The material was placed in methyl green and pyronin stain for three to five minutes (see below).

(3) Tissues rinsed in H_2O, and moisture removed by blotting.

(4) Slides were immersed in a differentiating solution of tertiary butyl alcohol and absolute alcohol (3:1) before they are completely dry, for two minutes or longer.

(5) Cleared in xylene and mounted.

Methyl green and pyronin stain was prepared by dissolving 0.5 gms. of methyl green in 100 ml. of O.IM acetate buffer at pH 4.4. This solution extracted repeatedly with

chloroform to remove residual methyl violet (sometimes as many as eight extractions may be necessary). Then 0.2gm. of pyronin B was dissolved in the solution of methyl green.

III. Recorcinol Blue.

Staining procedure. Three drops of the stock solution of stain (see below) were diluted with 10ml. of tap water. Microtomed material, after removal of the paraffin wax was taken down to water and stained in the above dye for two to five minutes, and directly mounted in a hygroscopic solution made by mixing equal volumes of potassium acetate (crystal), methanol and distilled water.

Preparation of dye:- 3 gms of recorcinol were dissolved (only fresh white material was used) in 200 ml. of distilled water, then 3 ml. of concentrated ammonia (at least 28% NH_4OH) were added and the mixture was heated for ten minutes on a steam bath (boiling avoided). The darkened reddish brown solution, stoppered with cotton, was kept at room temperature until a dark bluish colour appeared. It was then re-heated on the steam bath for about thirty minutes. The hot solution was filtered into an evaporating vessel and the heating continued until no significant amount of NH_3 escaped (checked with a moistened strip of red litmus paper in the steam zone). This stock solution was stored in a droper bottle.

(b) Details of the methods used for electron microscopy.

(1) Phosphate buffer.

(A) 3.58 gms of basic salt ($Na_2HPO_4.12H_2O$) dissolved in 1000 c.c. of distilled H_2O.

(B) 1.36 gms of acid salt (KH_2PO_4) dissolved in 1000 c.c. of distilled H_2O.

To obtain pH 7.0, solutions A and B were mixed in the proportion of 6:4.

(2) 1.5% gluteraldehyde.

To obtain 1.5% concentration, 6ml of 25% gluteraldehyde was diluted with 94 c.c. of phosphate buffer (pH 7.0).

(3) To make 4% OSO_4.

The capsules containing either 0.2 gms or 0.5gms of OSO_4 crystals were washed carefully in hot water to dissolve the crystals and settle down on the bottom, then washed in teepol to clean the outer surface. Washed again with distilled water to crystalize the liquid OSO_4 at the bottom of the capsule. The capsule was scratched carefully with a file, broken by holding it between filter paper. The bottom part was put in the stock solution bottle (that either contained 5 ml. or 12.5 ml. of distilled H_2O, according to the size of capsule used). The bottle was left at room temperature for an hour or so and then stored in the refrigerator.

Dehydration was carried out in the following ethanol series, allowing 30 minutes in each solution, 20%, 30%, 40%, 50%, 70%, 70%, 90%, 90%, and two changes of absolute ethanol.

This was followed by an ethanol and propylene oxide series, and material was kept for 30 minutes in each of the following:- (ethanol: p. oxide) 3:1, 1:1, 1:1, 1:3, 1:3, and then two changes of propylene oxide. Then material was carried through the propylene oxide and epon series, and kept in each mixture for about one hour.

(p. oxide: epon) 3:1, 1:1, 1:3.

The material was left in the last mixture for about twelve hours (overnight), then embedded in epon. After embedding the capsules were kept for about 24 hours at room temperature, then transferred to oven at 45°C for another 24 hours; and finally to an oven at 60°C, where they were kept for 48 hours or longer.

The epoxy resin epon was prepared as follows:-

Epikote	812	62ml	Mixture A.
DDSA		100ml	
Epikote	812	100ml	Mixture B.
MNA		89ml	

The ratio of A:B used is 7:3 and also 2:1.

Before using 1.5 - 2% of DMP30 (accelator) was added.

It was rather difficult to handle the small pieces of young stamens during the process of fixation and dehydration, so a small tube of perspex that had wire gauze on its two sides and could be opened into two halves, was used to carry the material from one solution to the other.

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Explanation of plates.

Plate I.

A - C. Face and side views of young spikes, depicting the acropetal order of development of spikelets. Two empty glumes (G) and successive lemmas (L) develop as lateral ridges on the spikelet axis. The floret primordia are seen as rounded papillae in the axils of young lemmas (Fp).
(A x 51, B x 43, C x 49 app.).

Plate 2.

A - C. Terminal spikelets with two acropetally developing glumes and successive lemmas. Each lemma substends a floret.

D. A portion of the spike, showing the lateral spikelets of similar age.
(A and B x 60, C x 60, D x 35 app.).

Plate 3.

An anterior view of a mature Triticum floret (lemma has been dissected out).

Plate 4.

A spikelet photographed from the top. Bottom floret, with three young stamens and a developing carpel, which is crescent shaped, and has partly enclosed the floret apex. (c)
Primordium of the second floret has flattened laterally, forshadowing the development of the two lateral stamens. (b)
The lemma which develops as a lateral crescent shaped structure is depicted in the top floret. (a)

Plate 5.

A - H. Different stages in the development of the carpel.

A - C. Earlier stages in the development, when carpel is crescent shaped, and partly enclosing the floral axis. (Photographed from the adaxial or posterior side).

D. Carpel with two distinct lateral projections, which are the primordia of two stigmas (st).

E & F. Later stages, in the development of the carpel.

G & H. Young carpels with carpellary and stigmatic hairs.

(A x 43, B x 30, C x 21, D x 32, E x 28, F x 27, G x 16, H x 15 app.).

Plate 6.

Vegetative leaves and glumes of various grasses of interest in connection with the interpretation of the Triticum carpel.

A. Secale cereale, L. Flag leaf seen in lateral view.

B. Same in dorsal view, (i.e. showing the future mid rib region).

C. Melica uniflora, Retz, showing the curious projection from the top of the "fused" edges of the leaf sheath.

D. Aegilops ovata L. glumes, each with four awns.

Plate 7.

A - C. Longisections of spikelets, representing the initial stages of lemma (A and B) at L' and L, floret primordium (B) at Fp, and of palea (C) and P'.

Plate 8.

A - C. Serial longisections of a floret primordium, illustrating the initiation of anterior stamen by periclinal division of subhypodermal and hypodermal cells. The initiation of the palea by periclinal division of a single hypodermal cell is also indicated at P'.

Plate 9.

A & B. Longisections (cut at right angles to the plane of glumes) of a developing stamen indicating the beginning of the filament.

C. Transection of a mature filament, showing thick walled intercellular spaces at Ics.

Plate 10.

A - C. Serial longisections (in the plane of glumes) of a floret primordium illustrating the initiation of the lodicule.

A. Median longisection of the floret primordium showing the developing anterior stamen, and the initiation of the lodicule by periclinal division of hypodermal cells at Lo'.

B. Longisection of the same floret (40 microns off the median) indicating the initiation of the lodicule.

C. Another longisection of the same floret (70 microns off the median) showing a periclinal division of a dermatogen cell in addition to divisions in the cells of hypodermis and subhypodermis.

Plate 11.

A - C. Longisections (at right angle to the plane of glumes) taken from different florets, to illustrate the continual initiation of the lodicules on the lateral sides of the floret at Lo' and Lo.

Plate 12.

A - C. Longisections (in the plane of glumes) from different florets, illustrating various stages in the development of the lodicule,(Lo).

Plate 13.

A - C. Transection of florets representing earlier stages in the development of the lodicules.

Plate 14.

Transections of young florets, illustrating lodicular traces (A at Lotr) and procambial strands (B and C) in the lodicules.

Plate 15.

A. Transections of basal portion of a mature lodicule, a and a' represent the magnified vascular strands of the same region.

B. Transection of the apical part of another lodicule; b, one of the vascular strands magnified.

Plate 16.

A 6 C. Longisections of young florets, depicting the various stages in the development of the carpel, (C', anterior portion of the carpel C", = posterior portion).

Plate 17.

A. Longisection (cut at right angles to the plane of glumes) of a young carpel at C.

B. Longisection (at right angles to the plane of glumes) of a young carpel, showing pattern of development of a stigma.

C. Transection of a fairly mature carpel, depicting the marginal growth on the inner face, for closing the top of the ovary.

Plate 18.

A - E. Serial transections of a fairly mature floret, depicting the presence of a projection on the posterior (or adaxial) side of the carpel, labelled as posterior projection (Pp).

Plate 19.

Serial transections of a floret, indicating the presence of a projection on the anterior (or abaxial) side of the carpel at Ast.

Plate 20.

A & B. Representing the initial stages in the development of stigmatic hairs, at Sth.

C. Transection of fairly mature stigmas and stigmatic hairs.

Plate 21.

A & B. Longisections of young carpels, depicting the initiation and early development of integuments.

C. Indicating similar stages in the development of the integuments in a transection of a carpel.

D. Longisection of a fairly mature carpel showing micropyle.

Plate 22.

A - D. Serial transections of young florets, indicating the position of origin of procambial strand in the lateral stamen (Ls).

Plate 23.

A & B. Transections of the carpel (24 microns apart) illustrating the position of origin of procambium in the future median strand. These sections also show continued initiation of the carpel on the posterior (adaxial) side of the floret axis.
(Note: The periclinal division in the dermatogen cells at the posterior regions).

Plate 24.

A - D. Serial transections of young florets, illustrating the position of origin of procambium in the future median and lateral strands of the carpel.

Plate 25.

Transection of developing vegetative shoot of Triticum, illustrating the differentiation of first three procambial strands (at Pcst) in the last formed leaf.

Plate 26.

A - C. Transections of young florets, illustrating the position of origin of first protophloem element in the anterior stamen (Pph).

Plate 27.

A - C. Slightly later stage in the differentiation of the first protophloem element in the anterior stamen.

Plate 28.

A - C. Serial transections of a developing floret, illustrating the position of origin of first element of protoxylem (pxy).

Plate 29.

A - C. Serial transections of a developing floret, depicting the position of origin of first protophloem element in the median procambium of the carpel.

Plate 30.

A - F. Serial transections of a mature carpel, illustrating the differentiation of protophloem and protoxylem elements in the funicular strand (Fst).

Plate 31.

A - C. *Avena sativa*, serial transections of a young floret, illustrating the differentiation of procambial strand in an anterior stamen.

Plate 32.

A - C. *Avena sativa*, serial transections of a young floret, illustrating the differentiation of procambial strand in the future median strand of a carpel.

Plate 33.

Transection of differentiating protophloem elements of the stamen (measured about 500 microns),(x10,000 app.).

Plate 34.

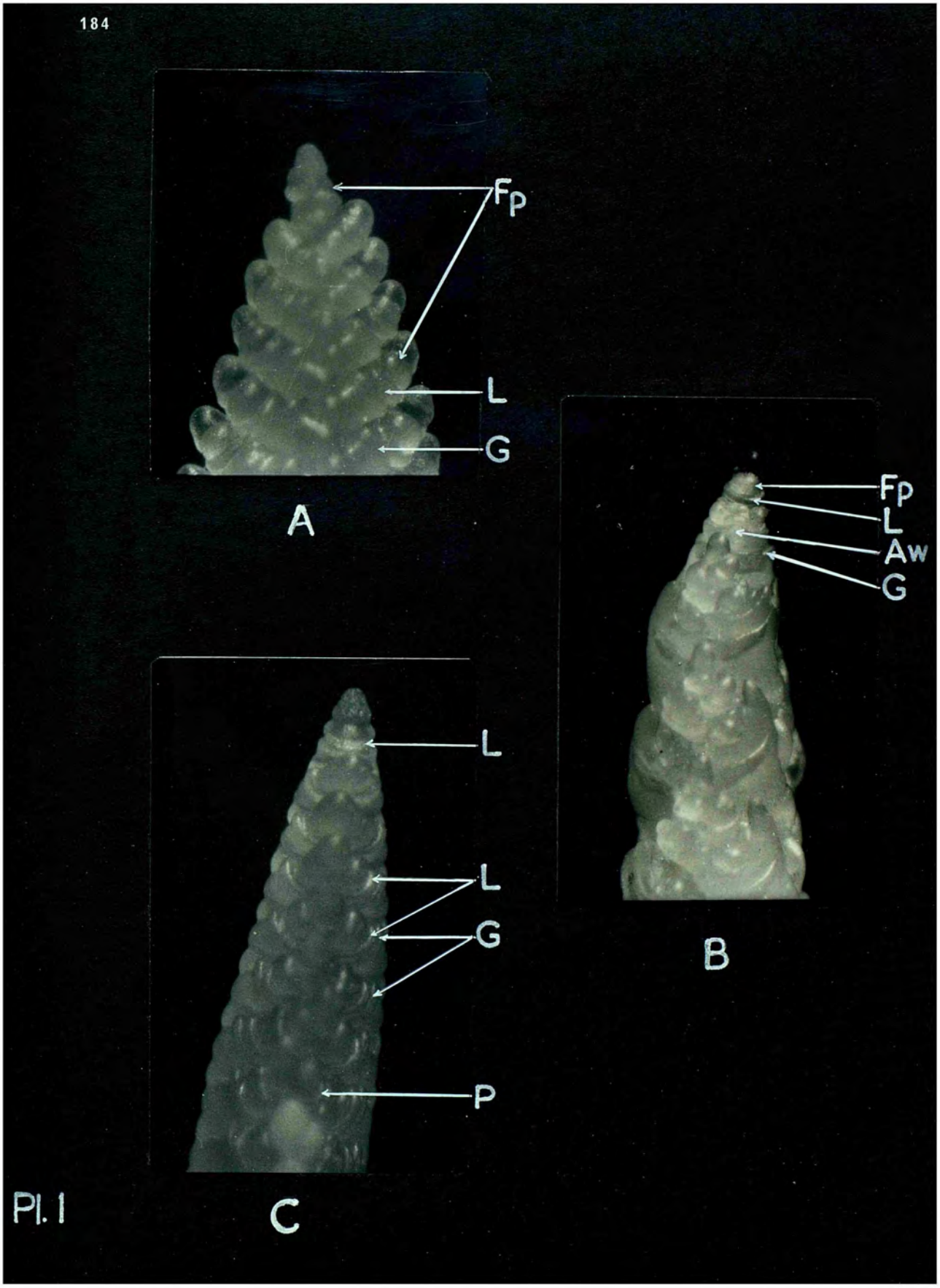
One of the protophloem elements(shown in Plate 33) is magnified (x20,000 app.).

Plate 35.

A later stage in the differentiation of a protophloem element of the stamen as seen in transection (x10,000 app).

Plate 36.

Transection of a differentiating protophloem element of the stamen (measuring about 800 micron) (x 15,000 app.).



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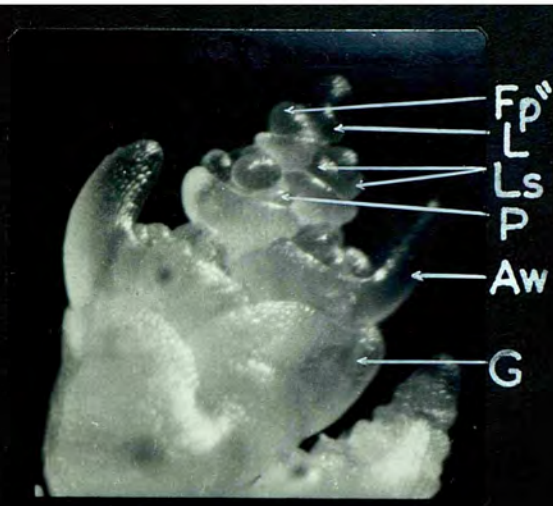

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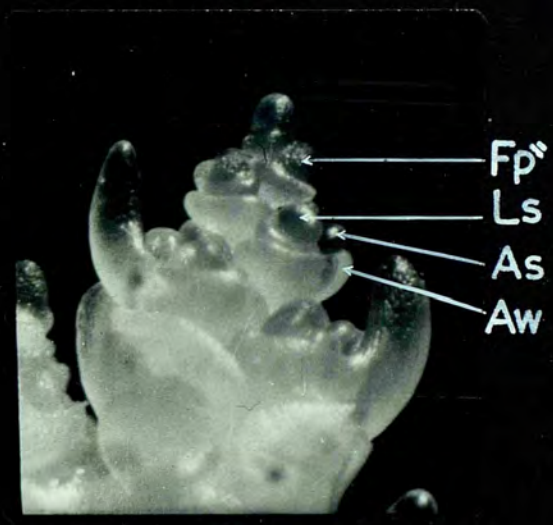

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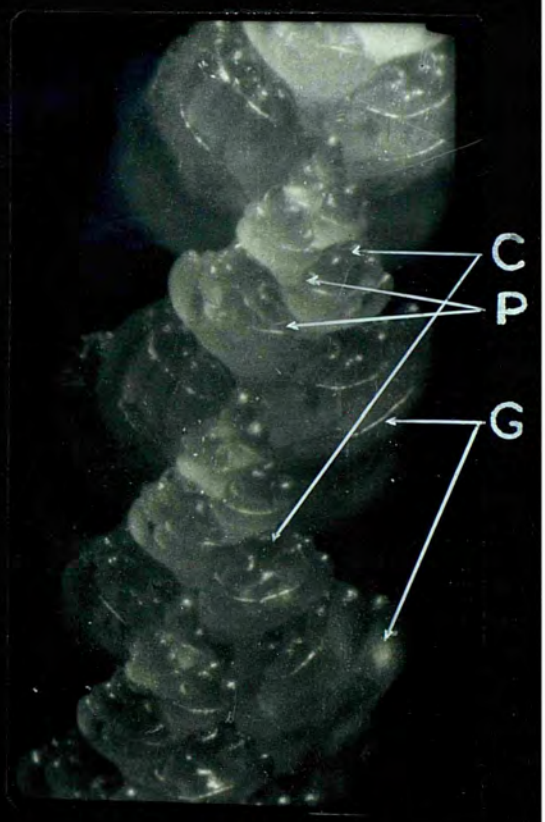

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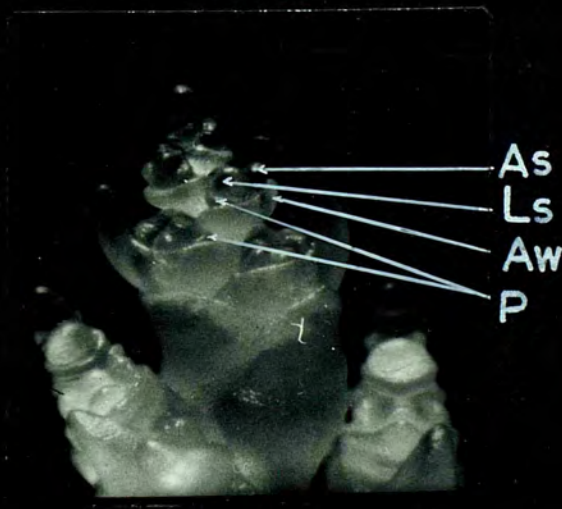
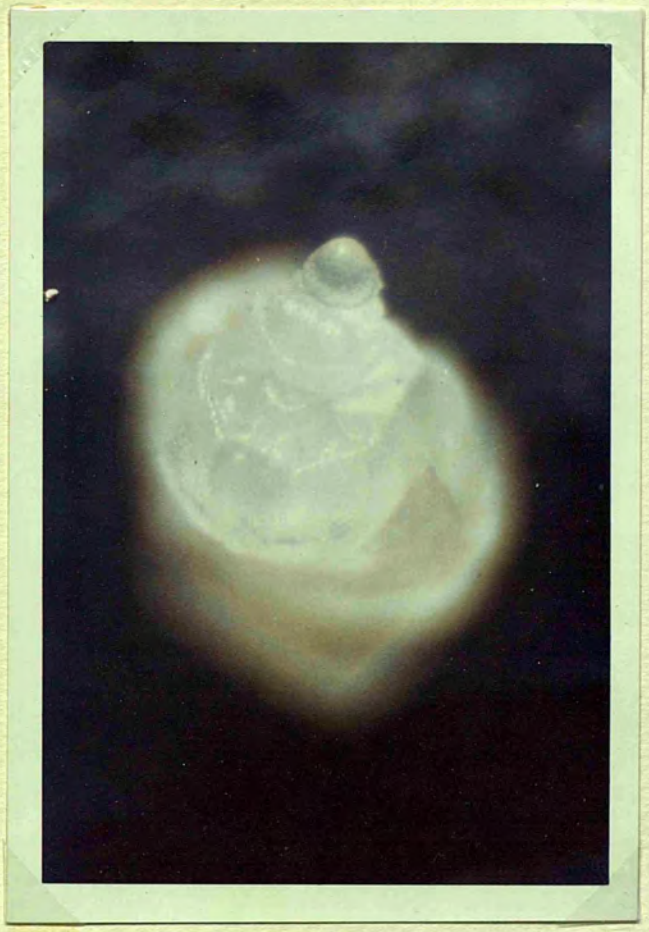


C

Pl. 2

Pl. 3

R.4



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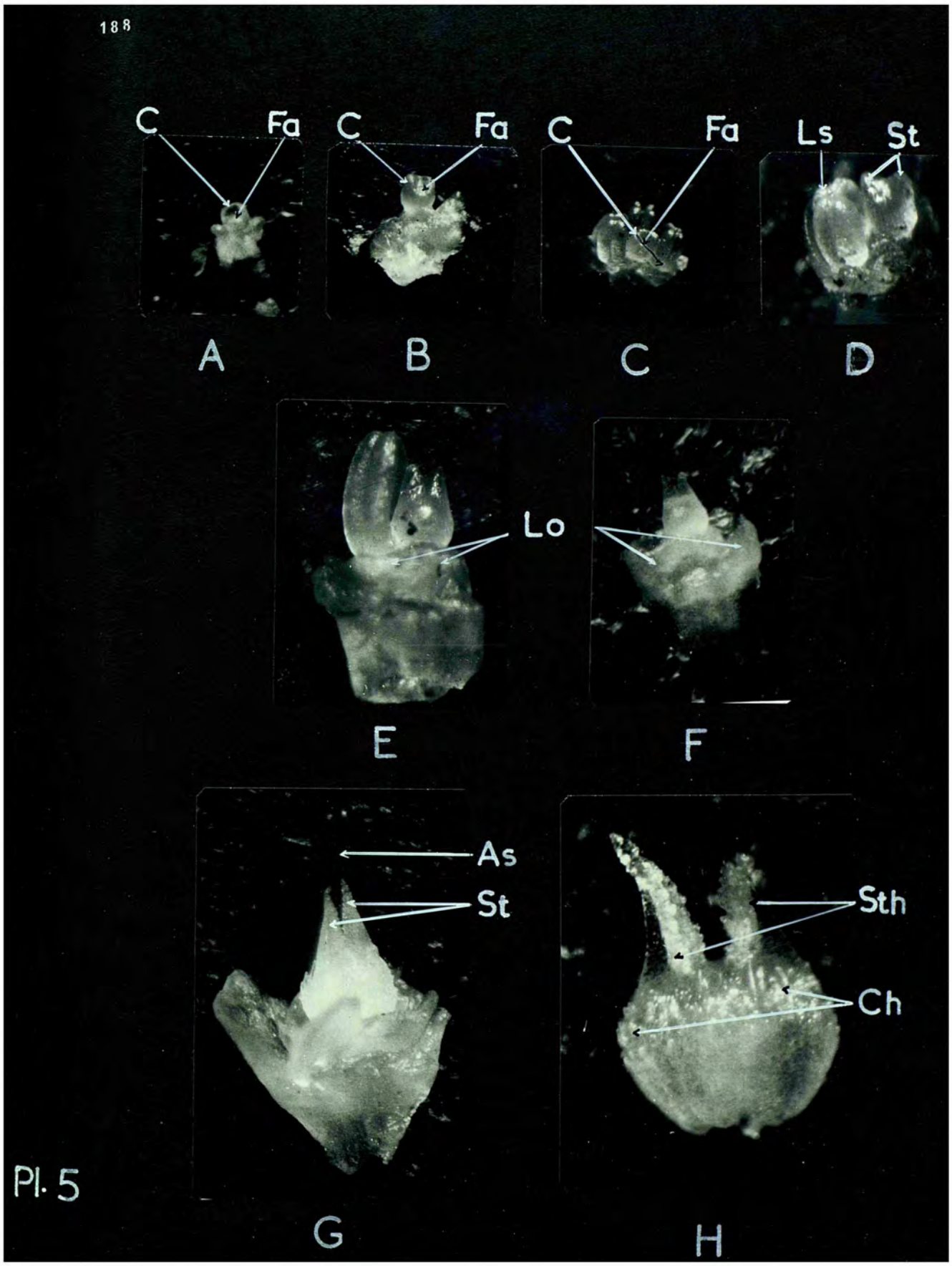
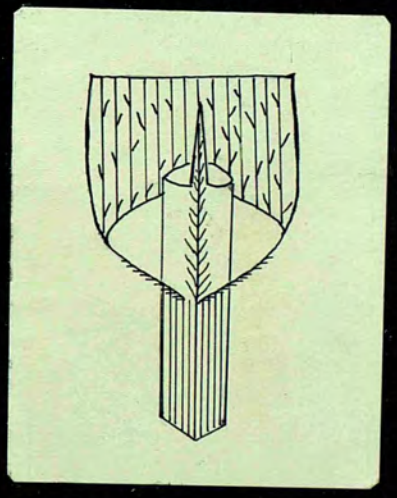


Pl. 5

A

B



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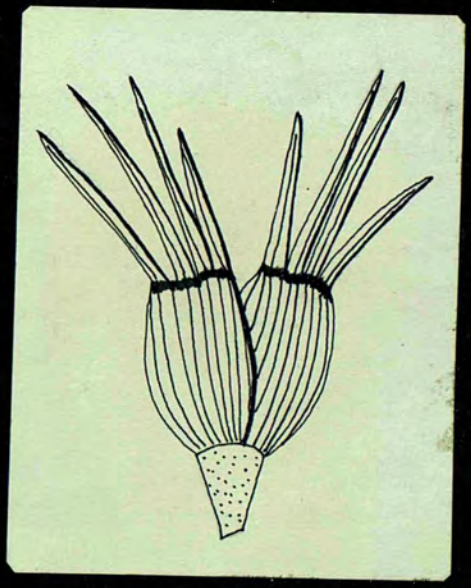

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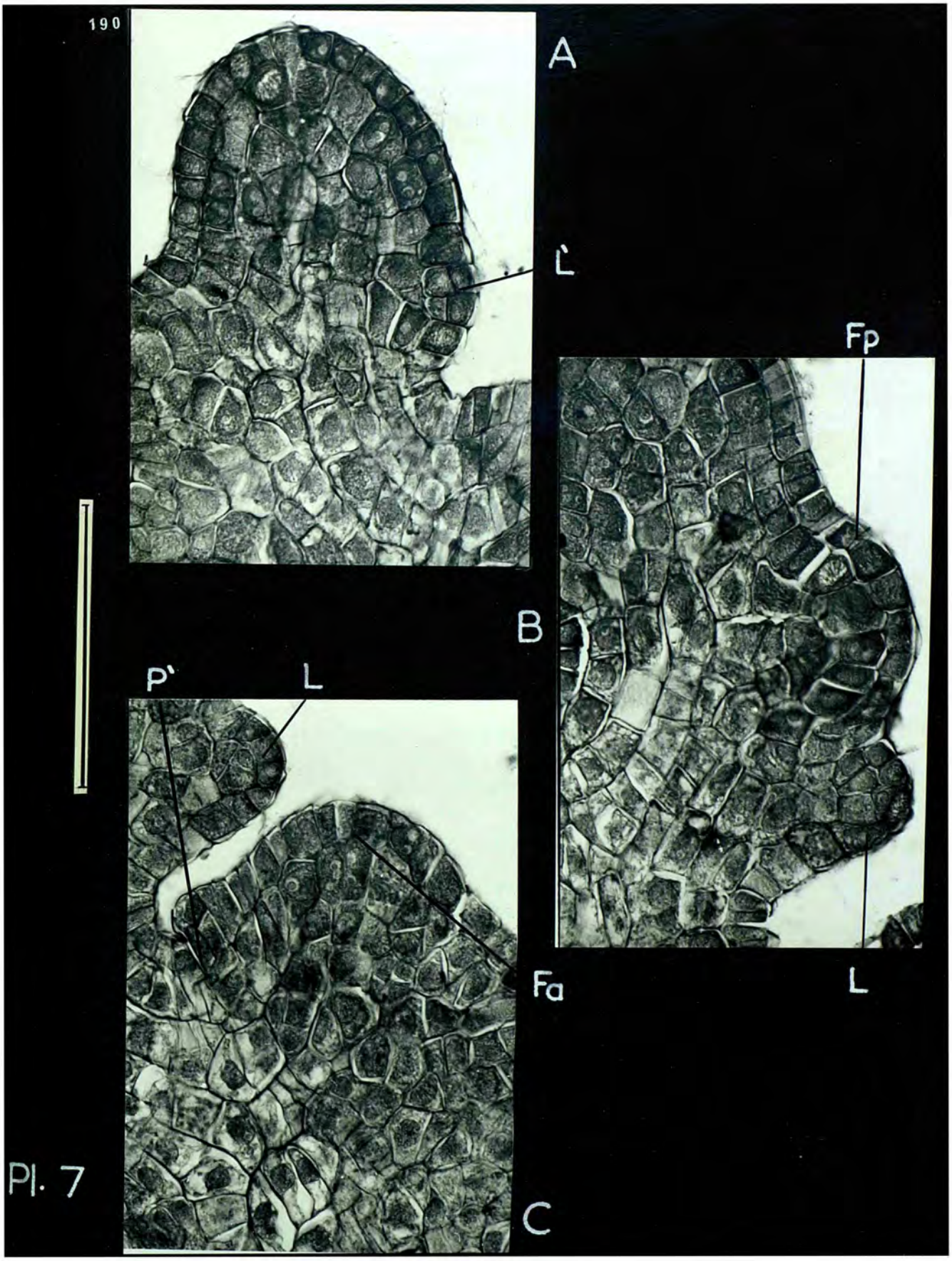
Pl. 6



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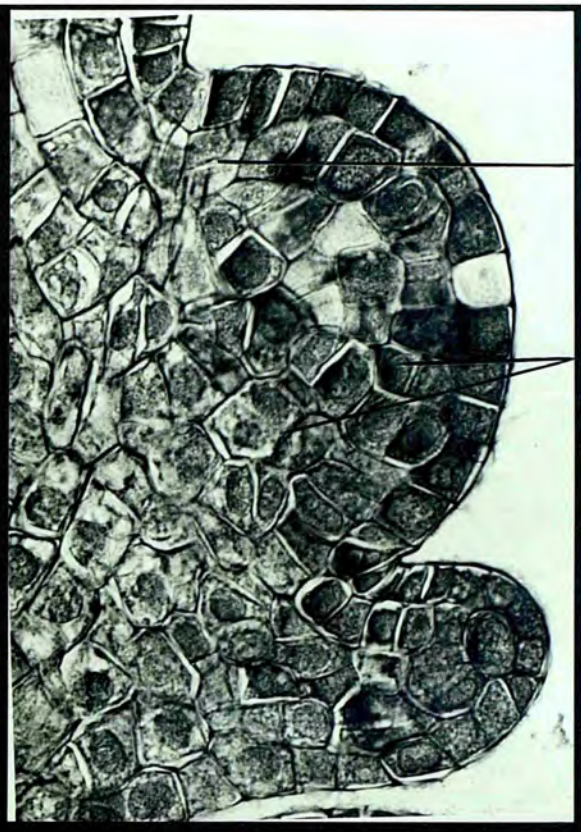
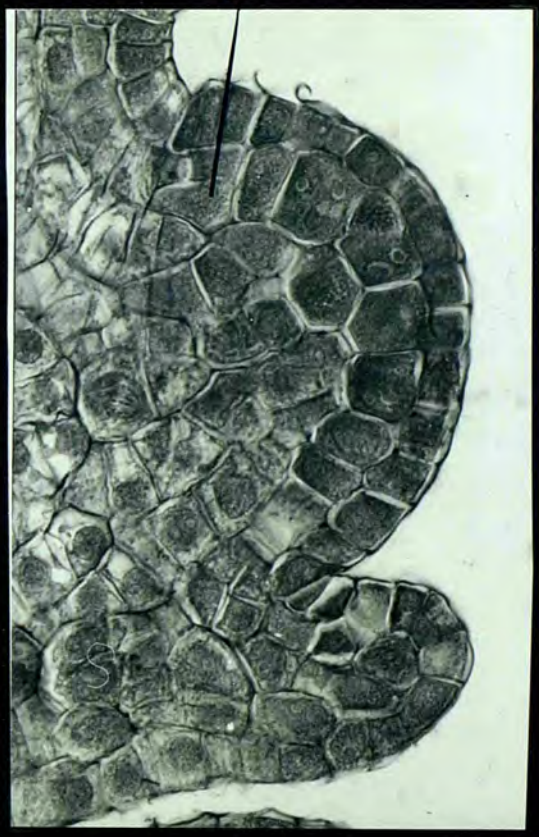
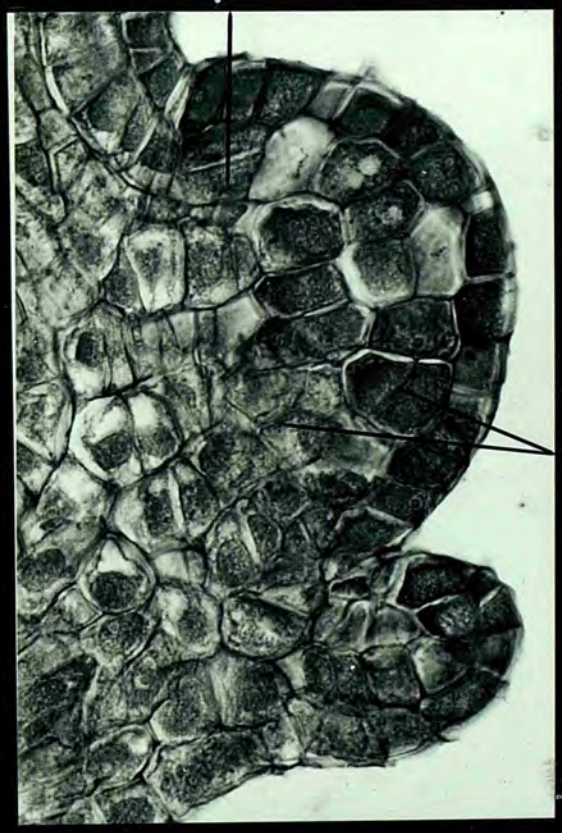
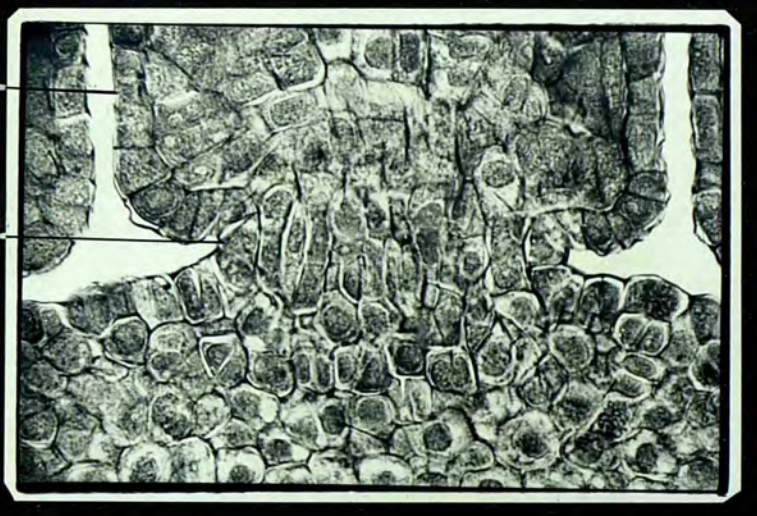






Pl. 8

A



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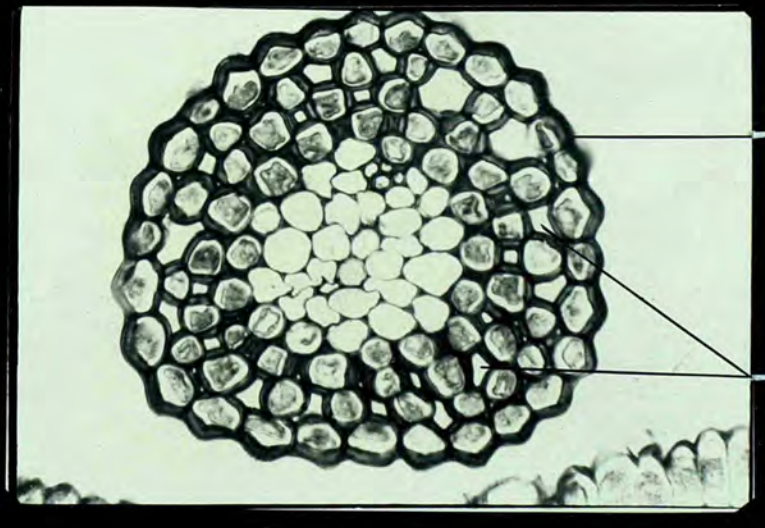


C

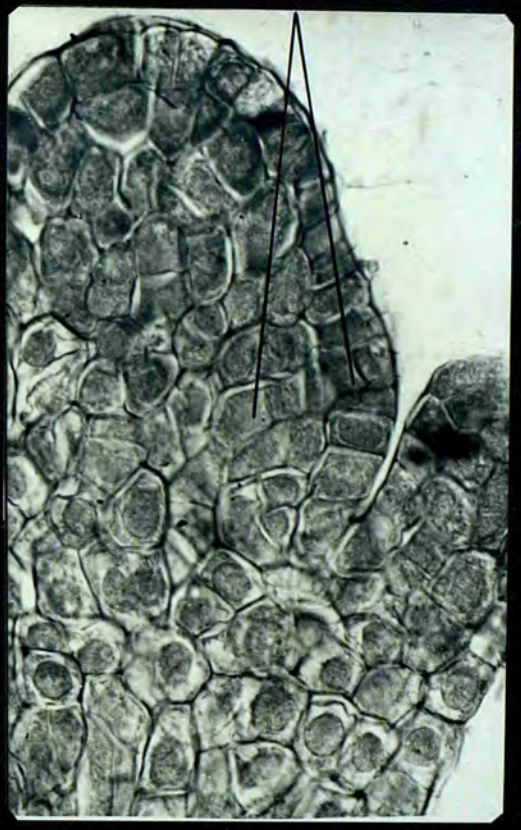
Pl. 9

A

As

Lo'

Lo'



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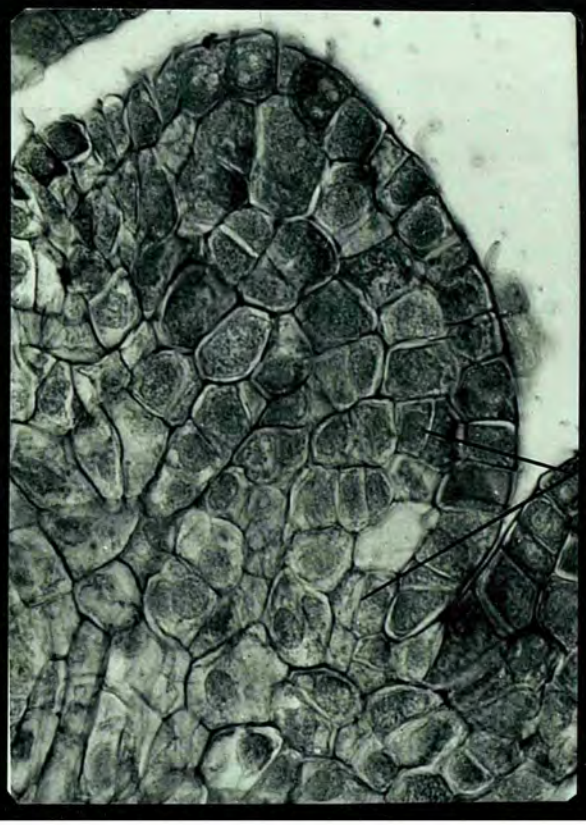
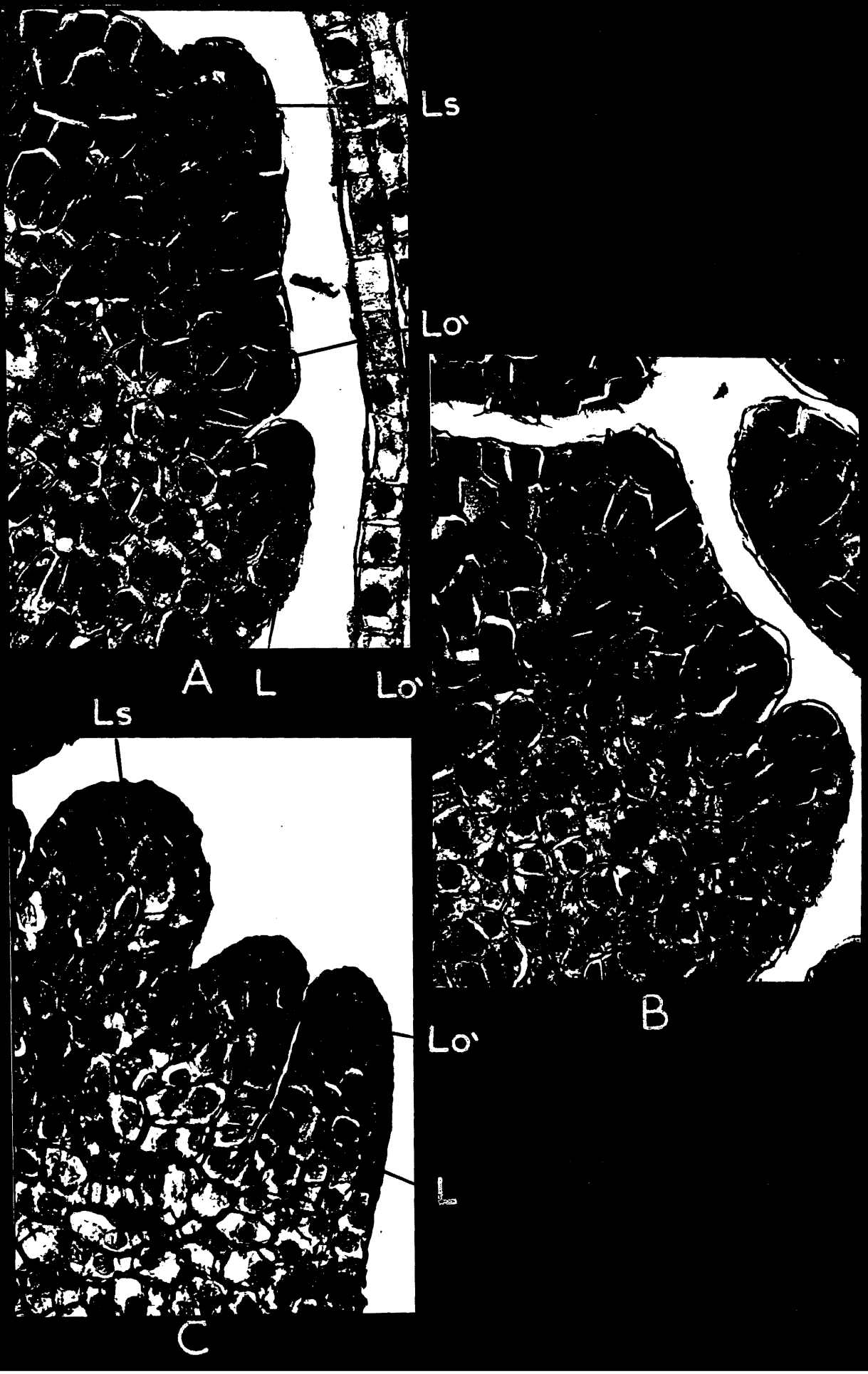


Lo'

B

Pl. 10



Pl.11

A

Lo

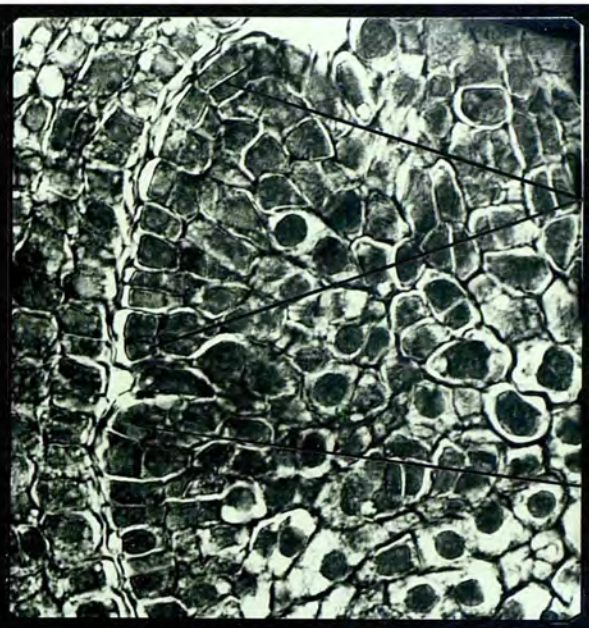
B

Lo

Lo

C

PI·12



A

Lo

P

L

Lo

P

B

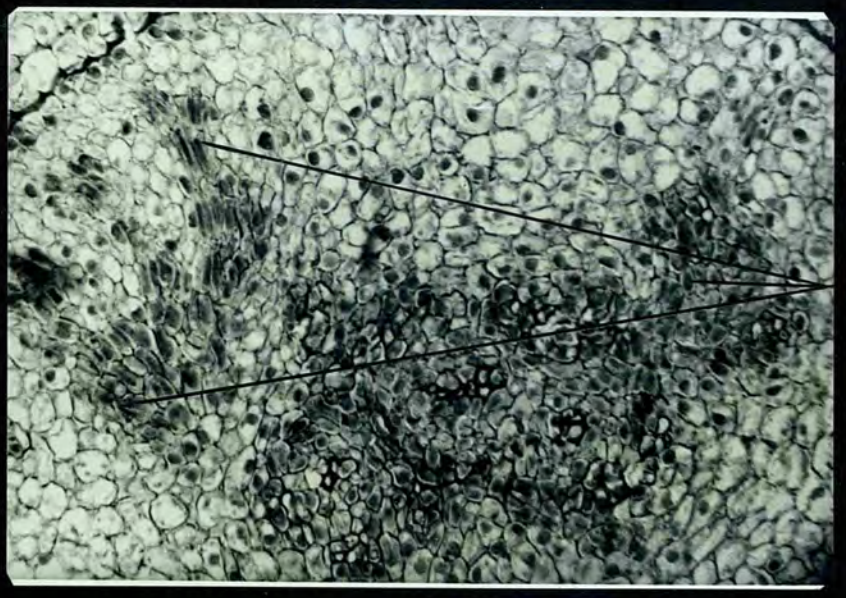
L

Lo

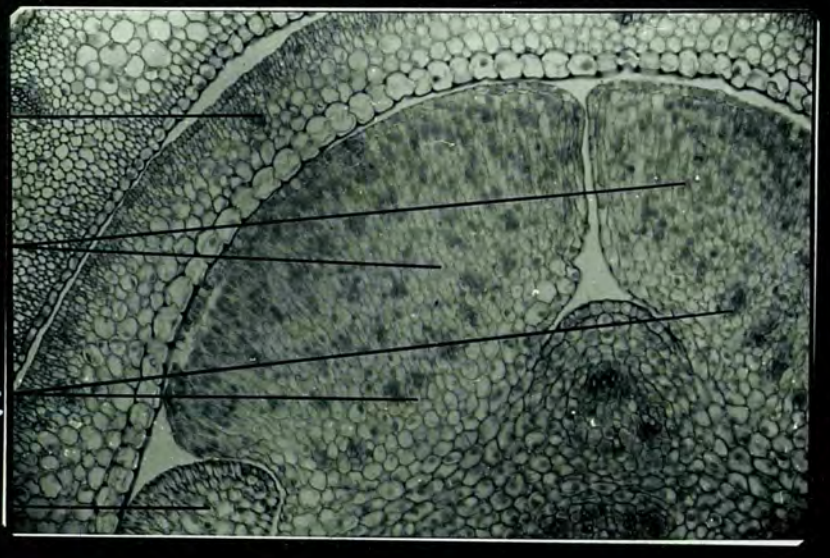
P

C

Pl.13



Lotr A



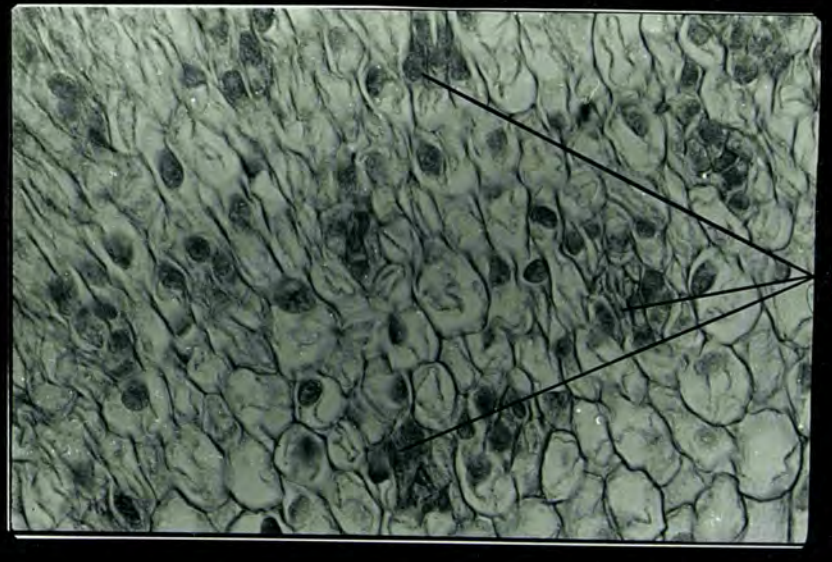
L

Lo

B

Pcst

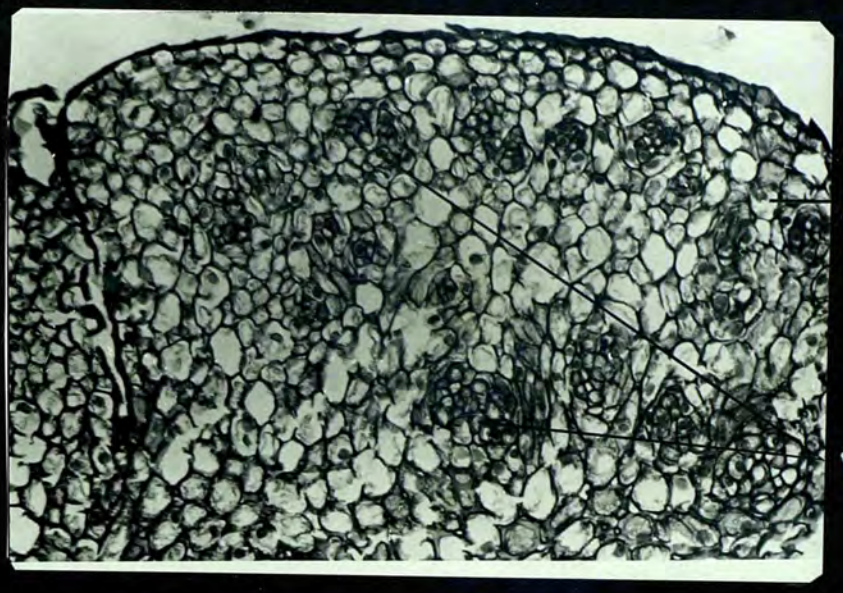
P



Pcst C

A

Pl.14



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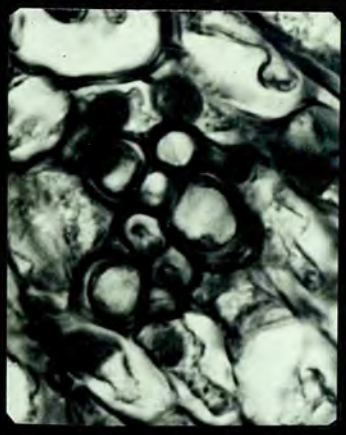

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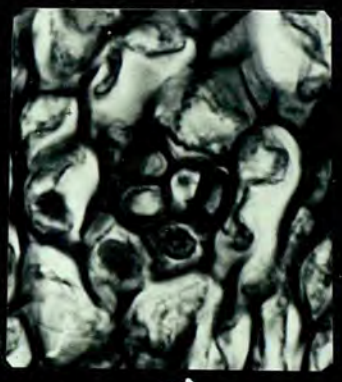

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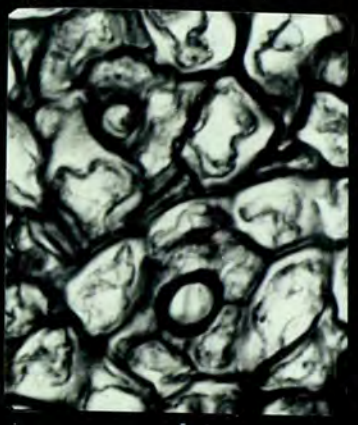

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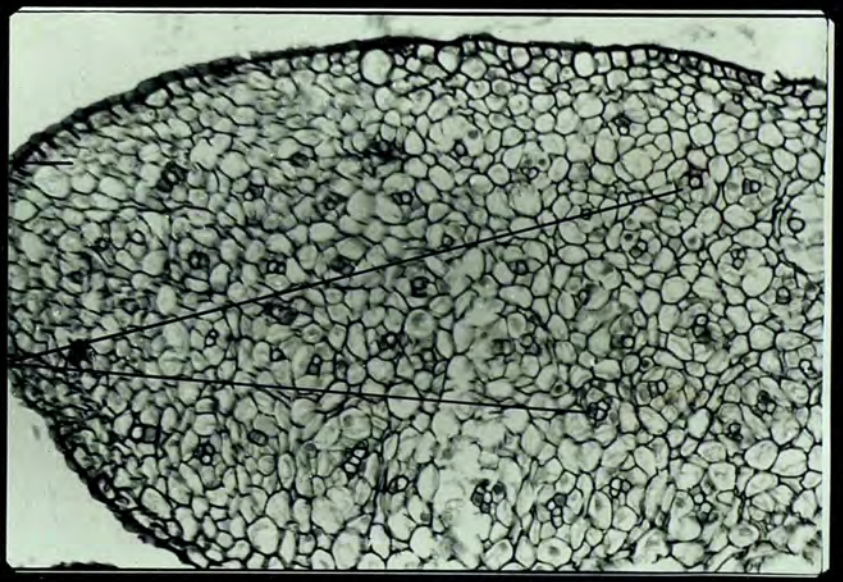


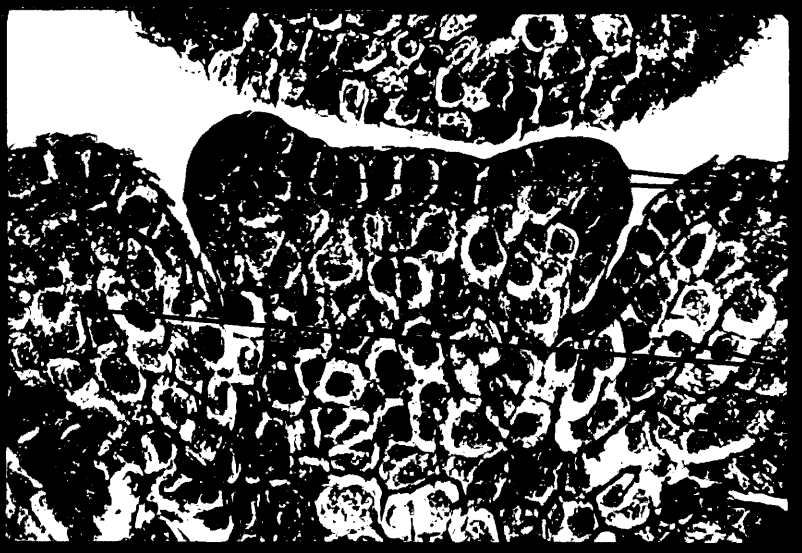
B

Pl.15

Pl.16

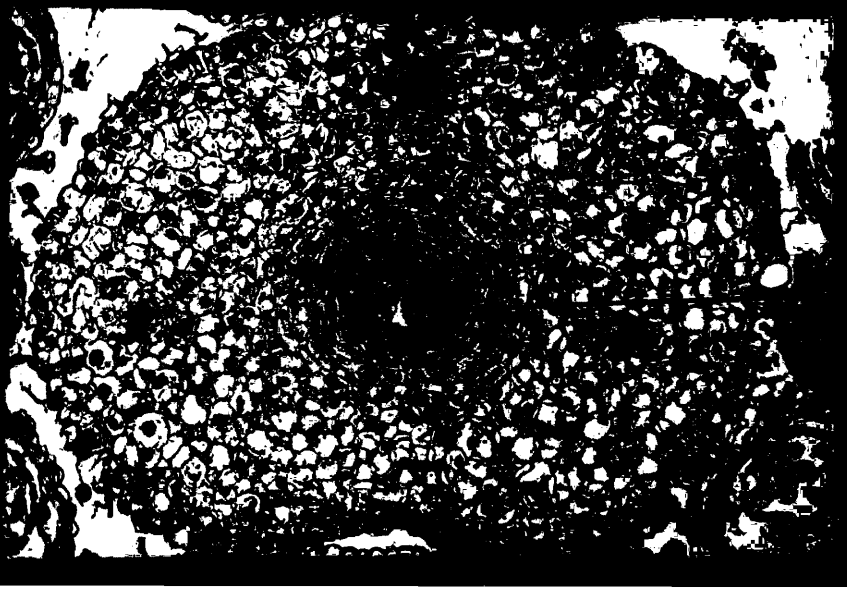
200

A



B

C



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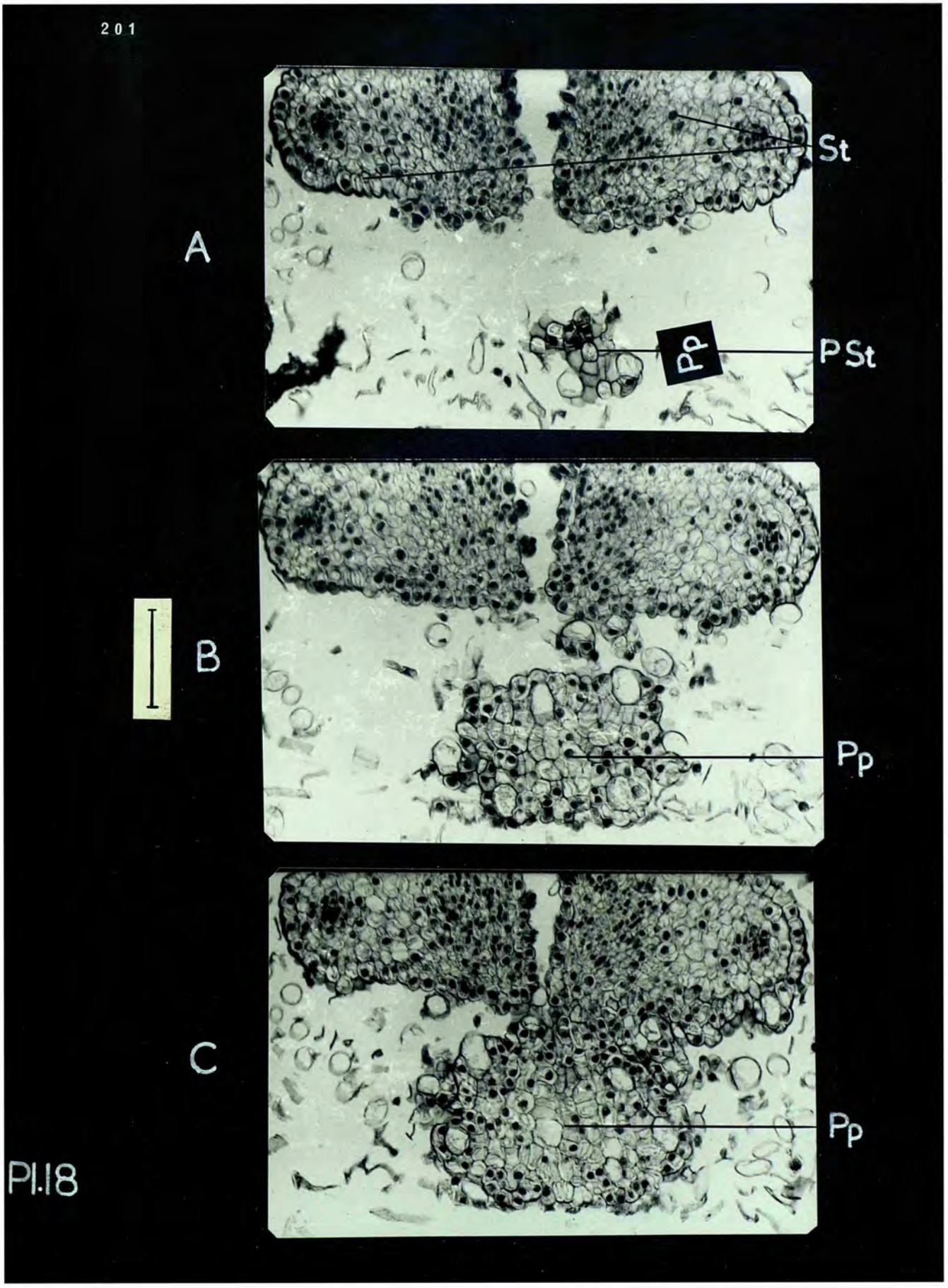

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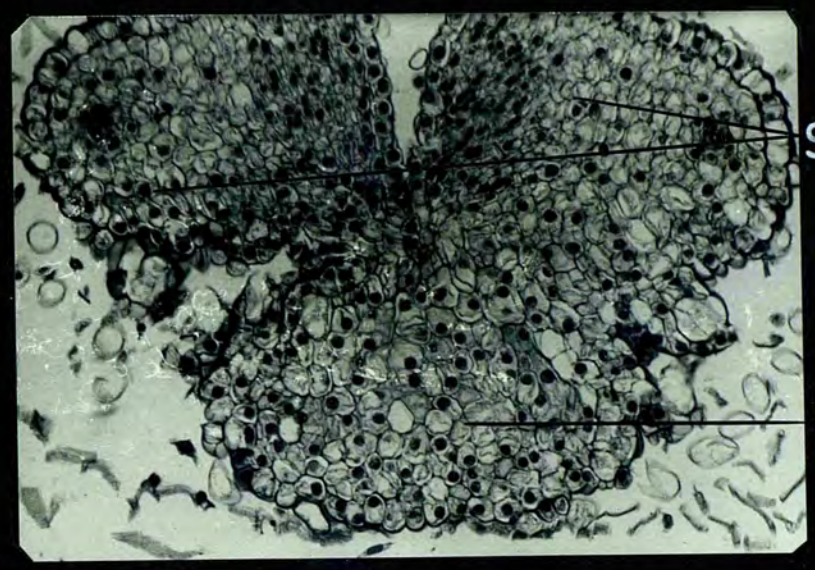

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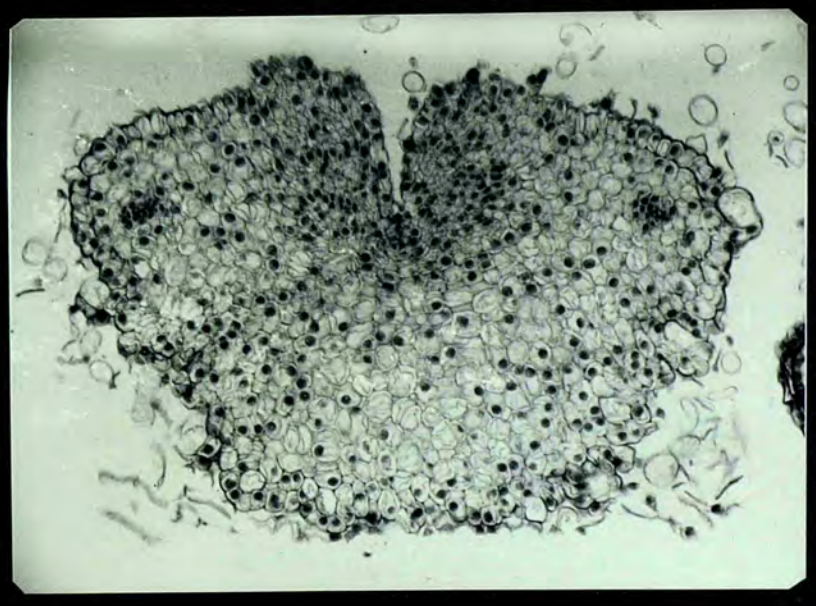

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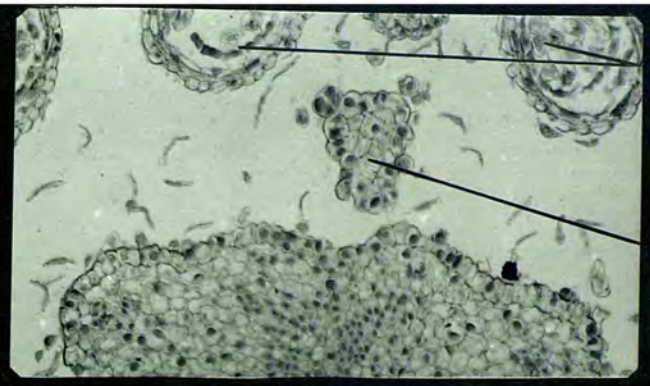
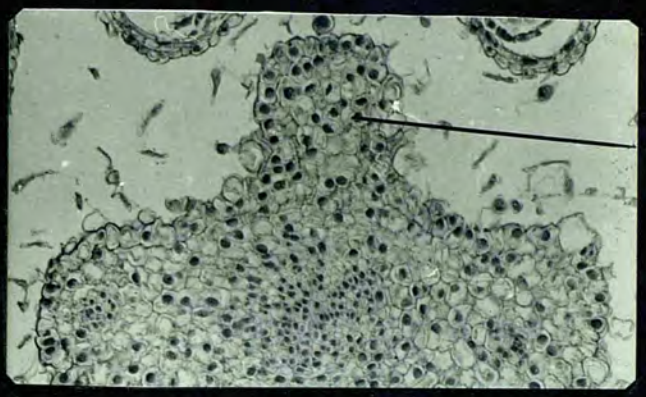
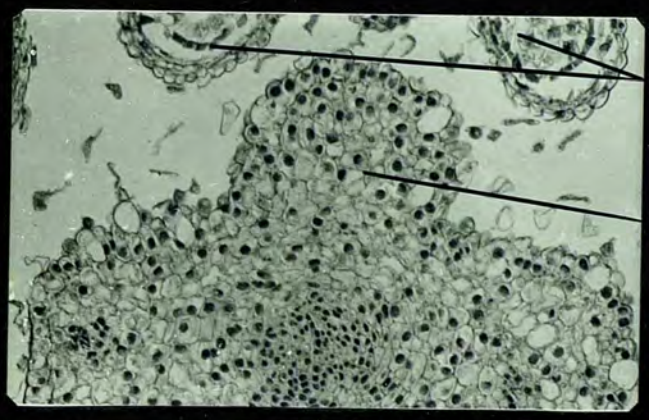
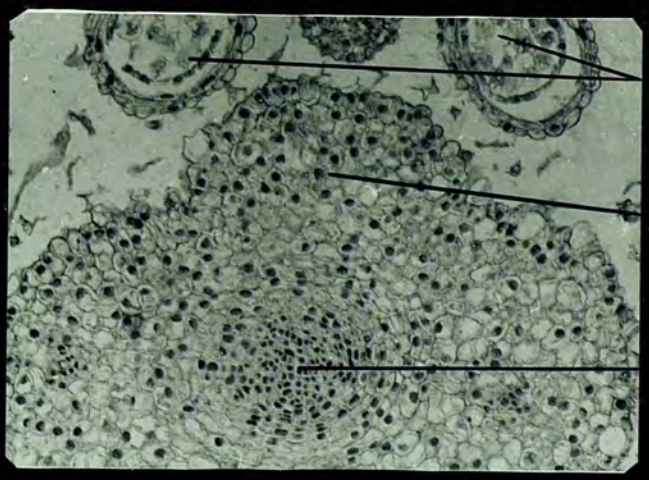
Pl.18









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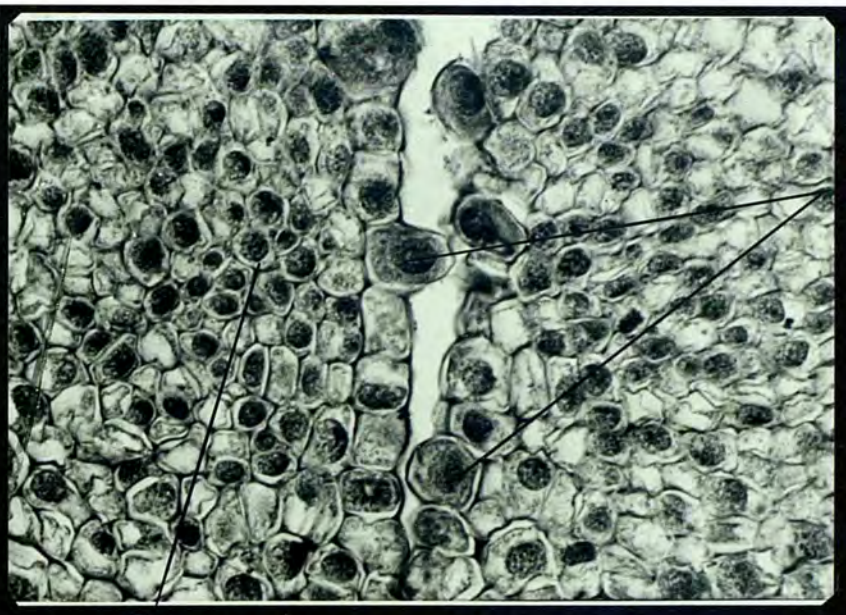

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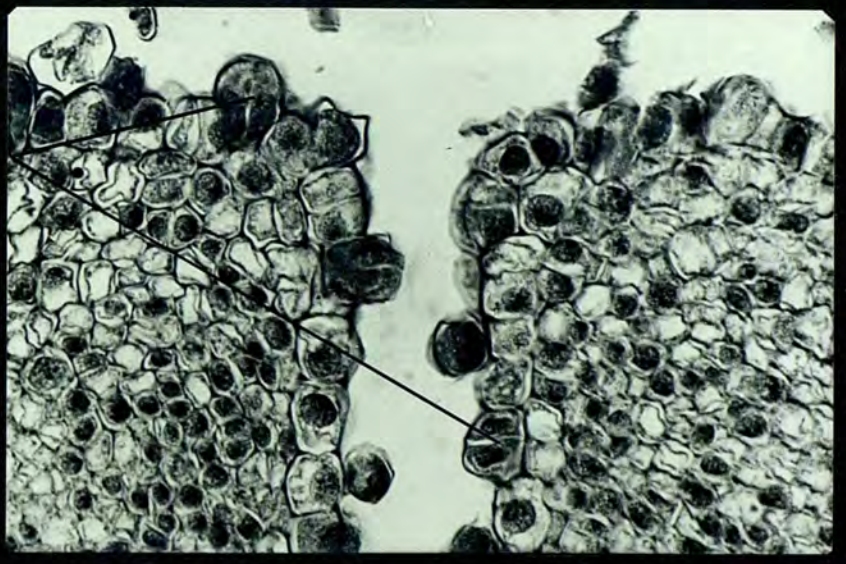
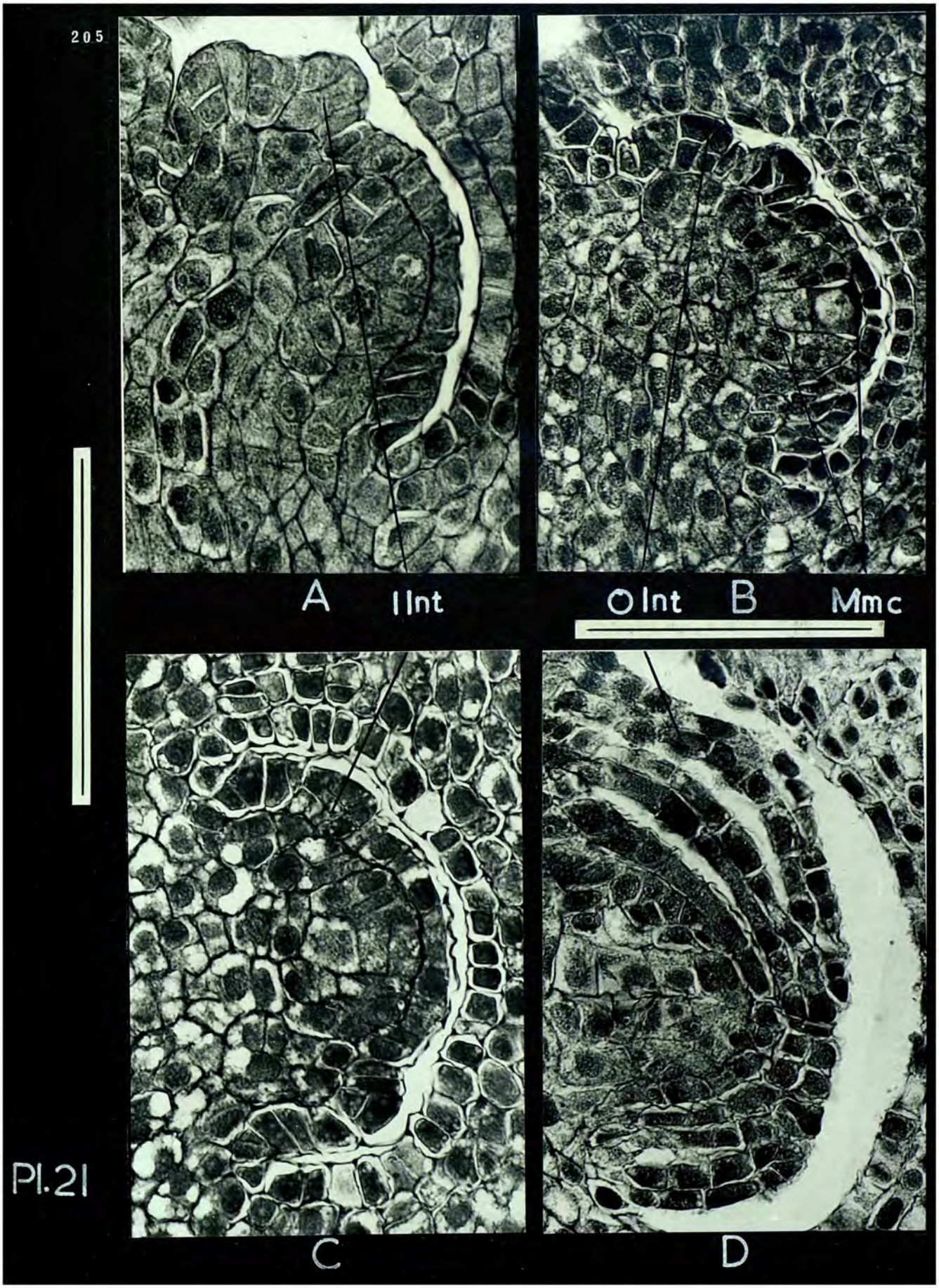


B

C

Pl.20



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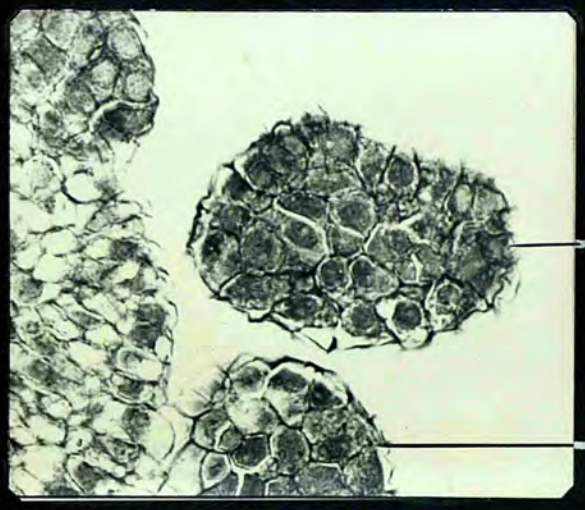

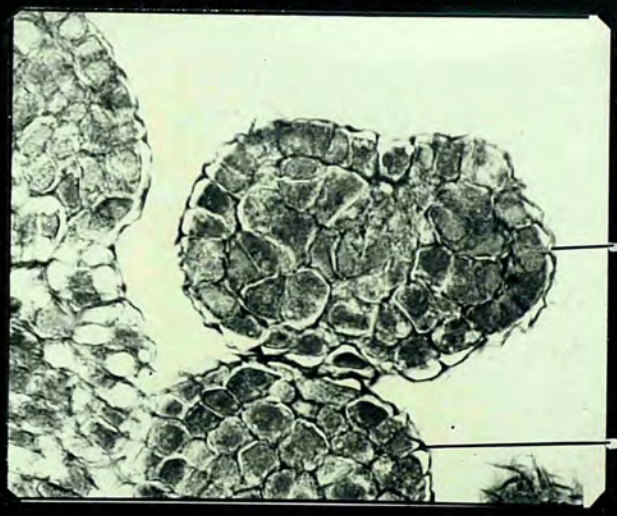

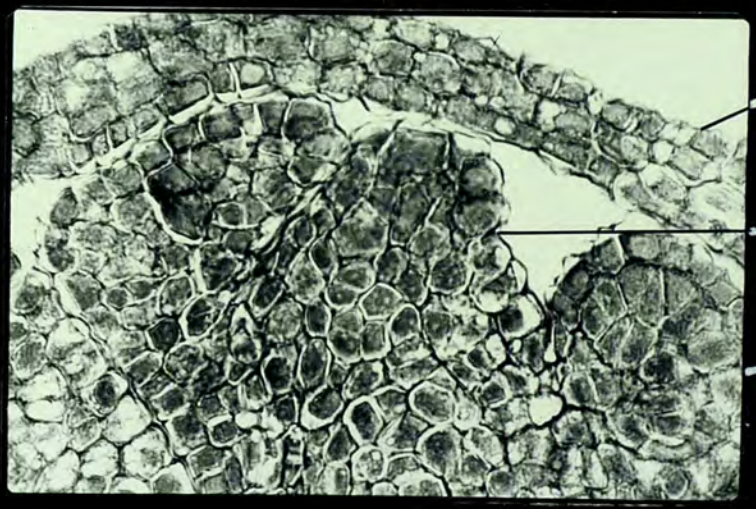

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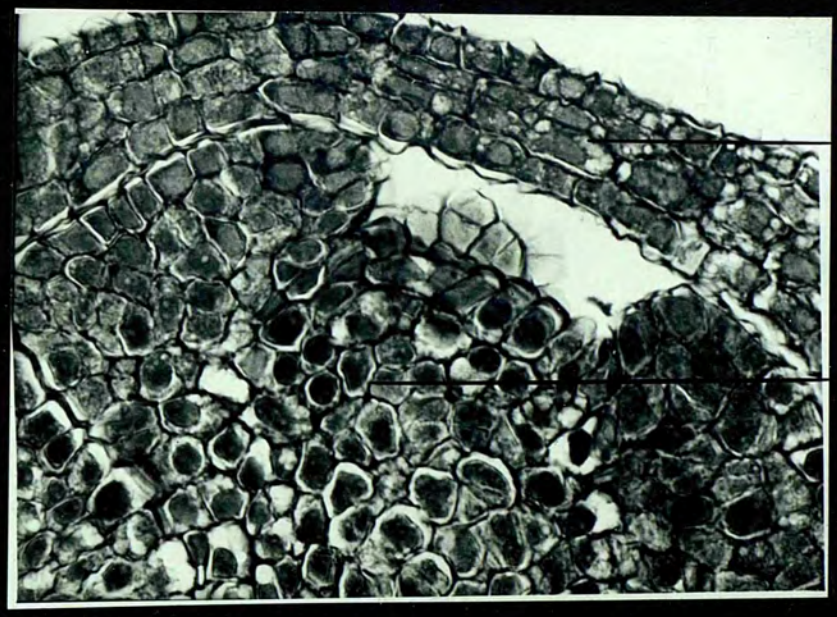

Pl.22

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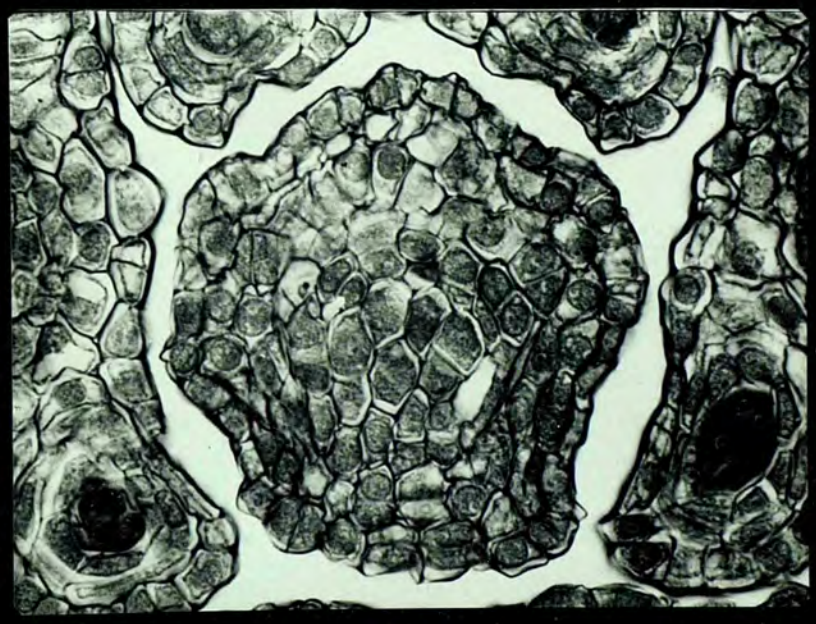

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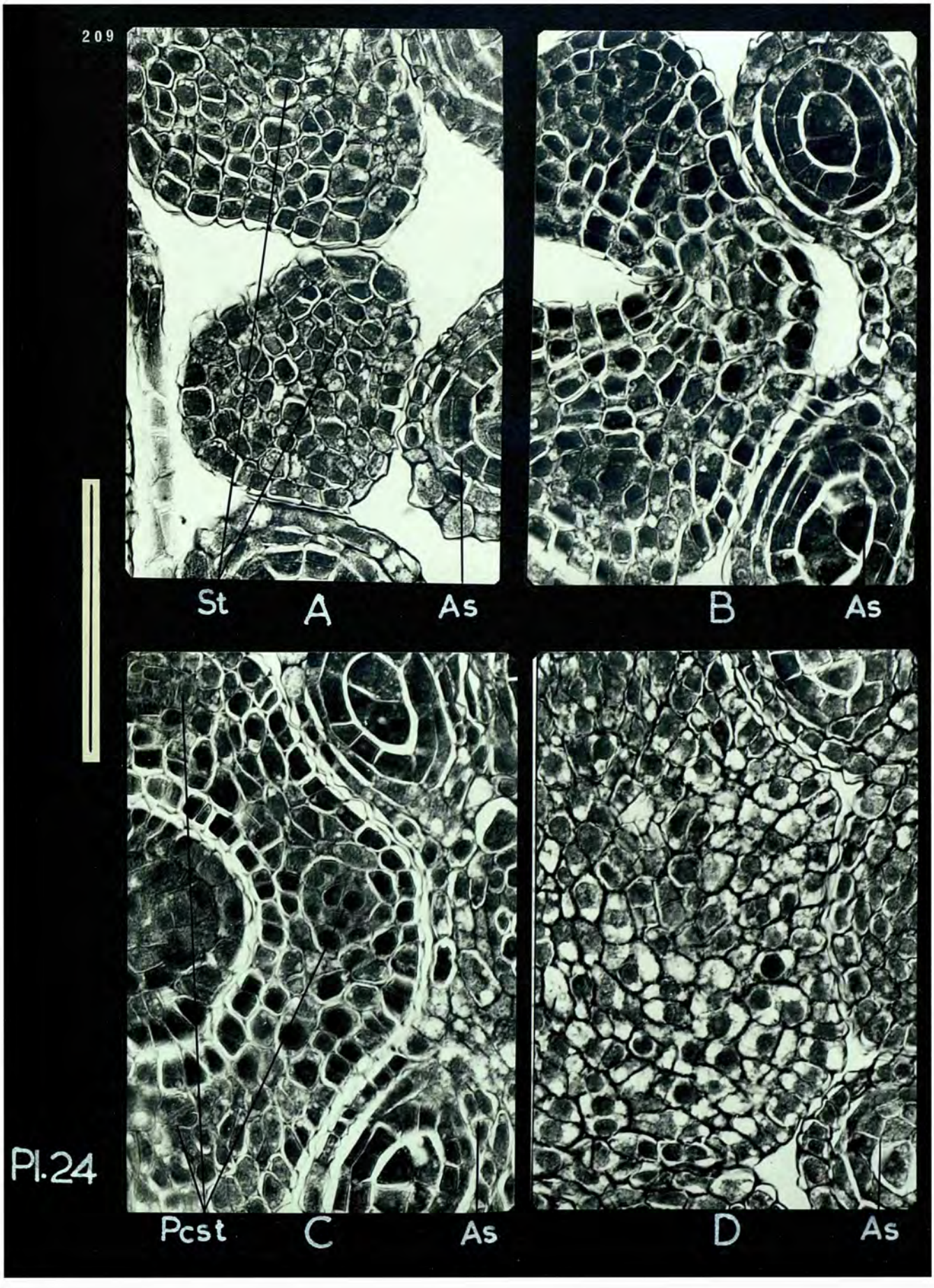
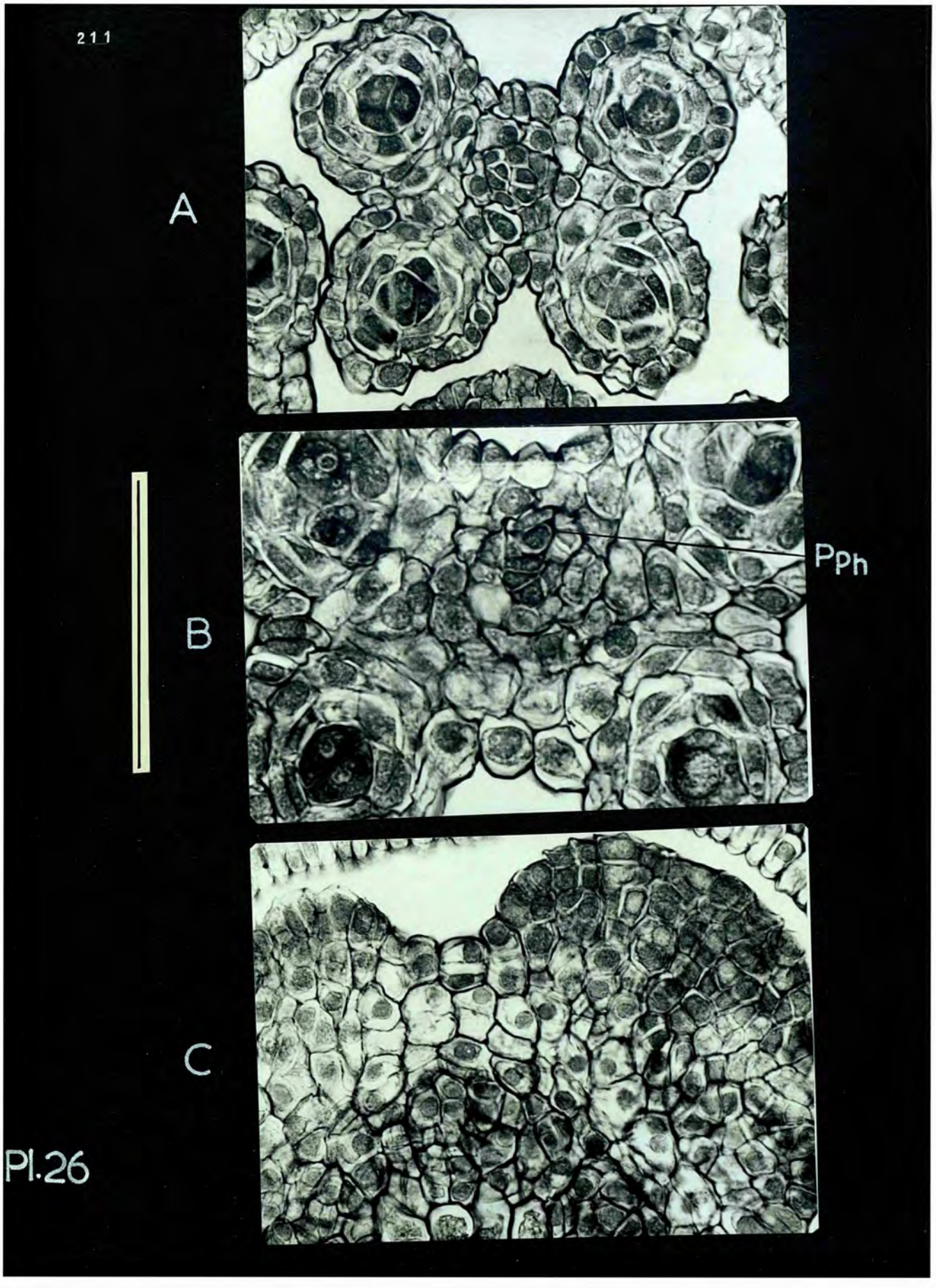
Pl. 23

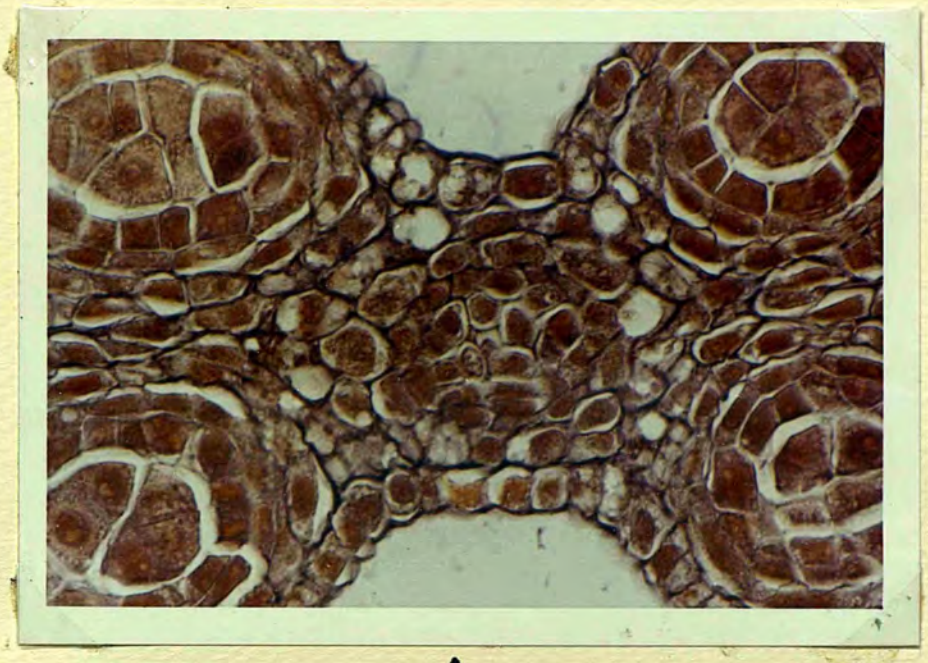


Pl.24

Pl.25



Pl.26



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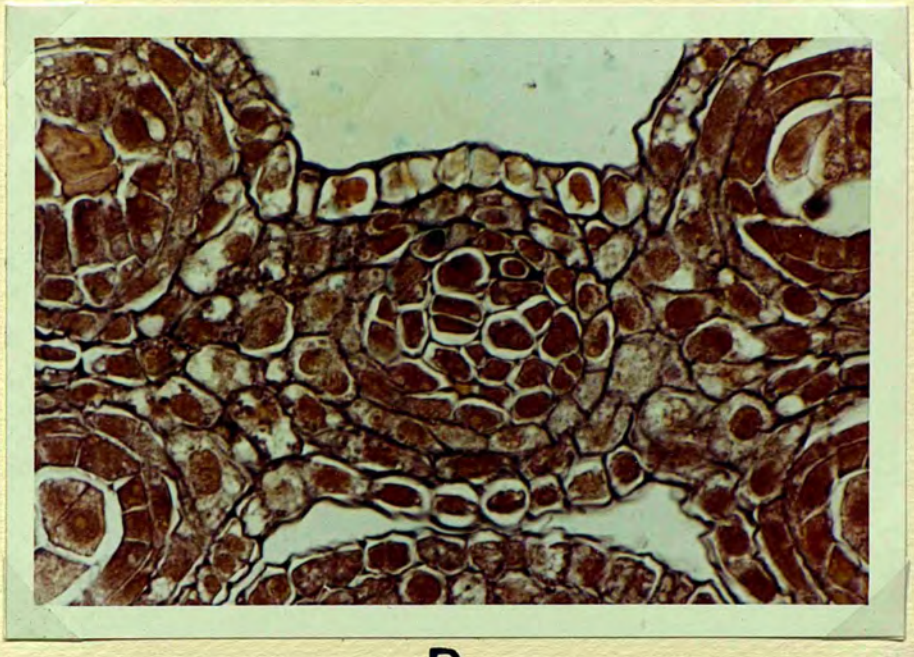
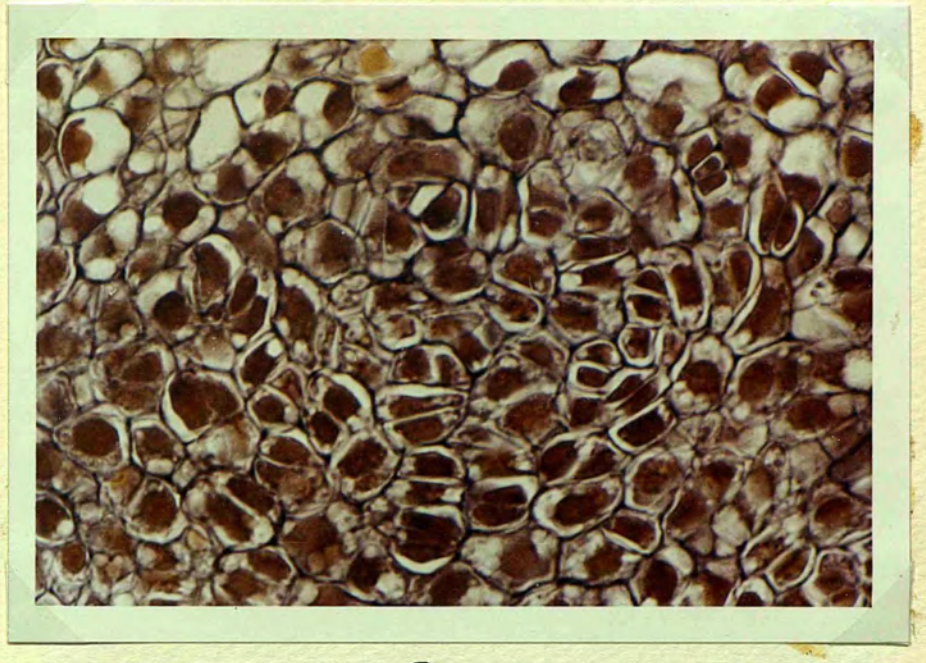


B

Pl.27



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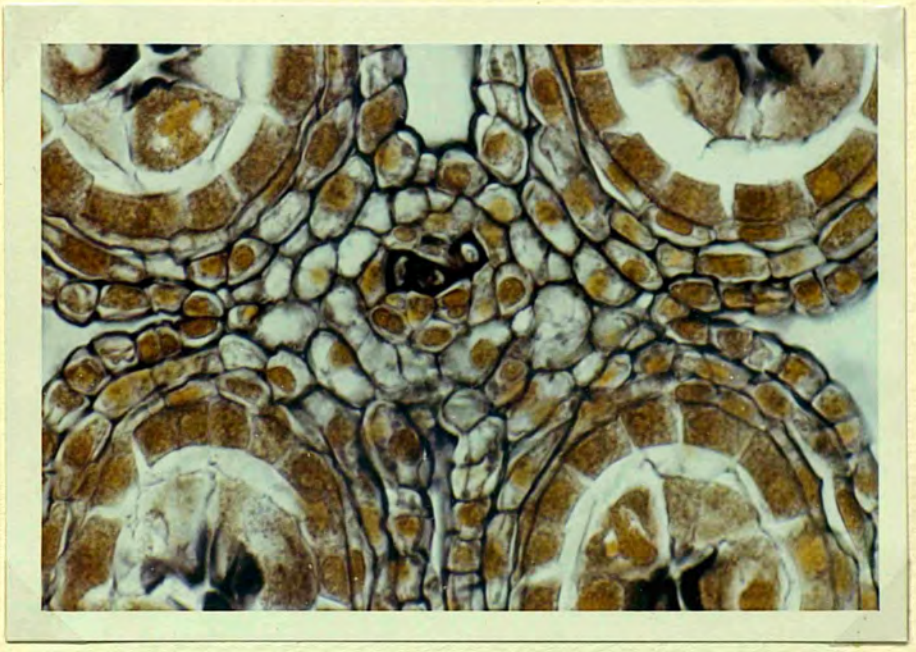
Pl.27



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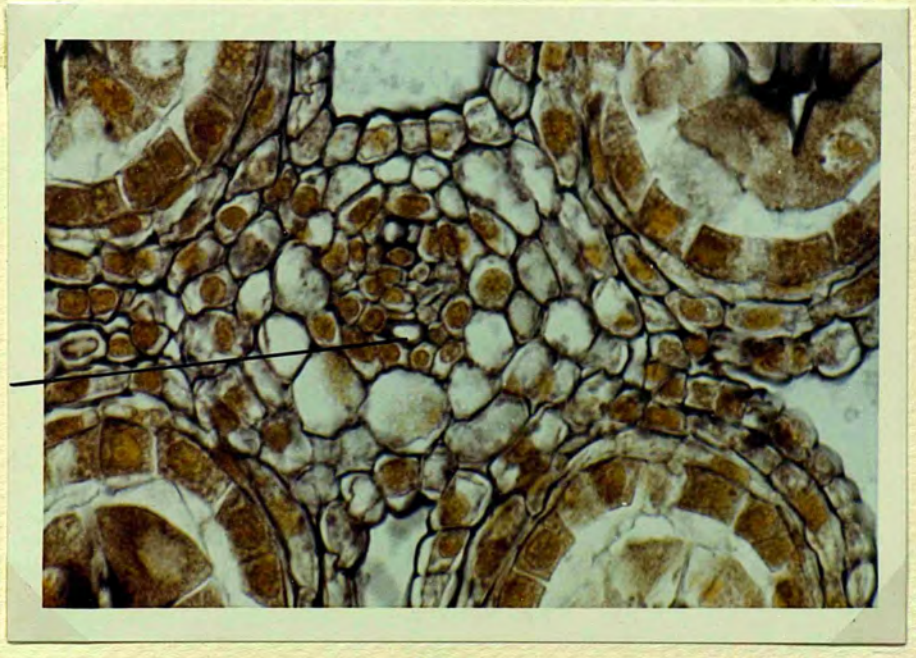
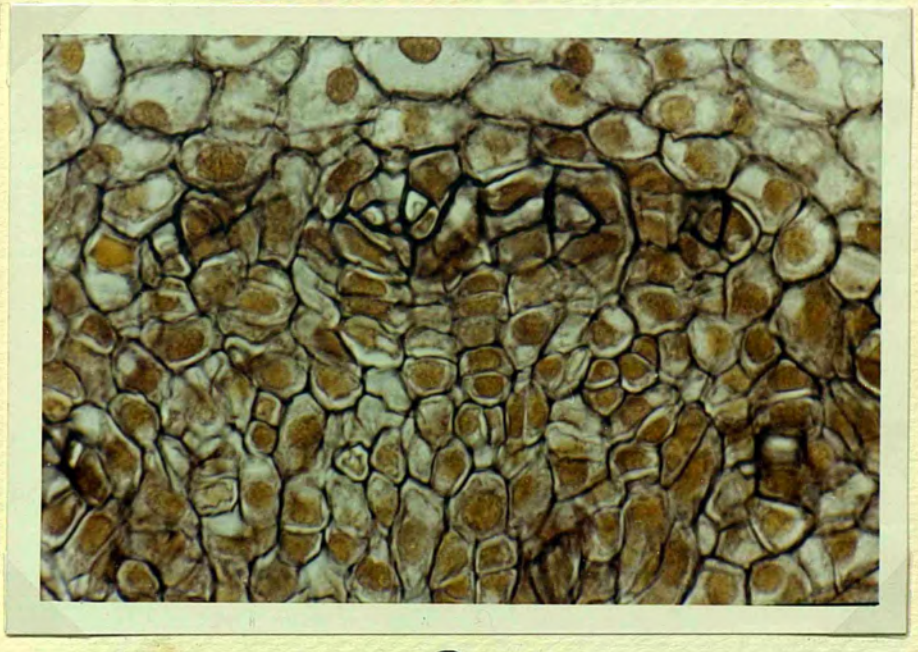


Pxy

Pl.28

B



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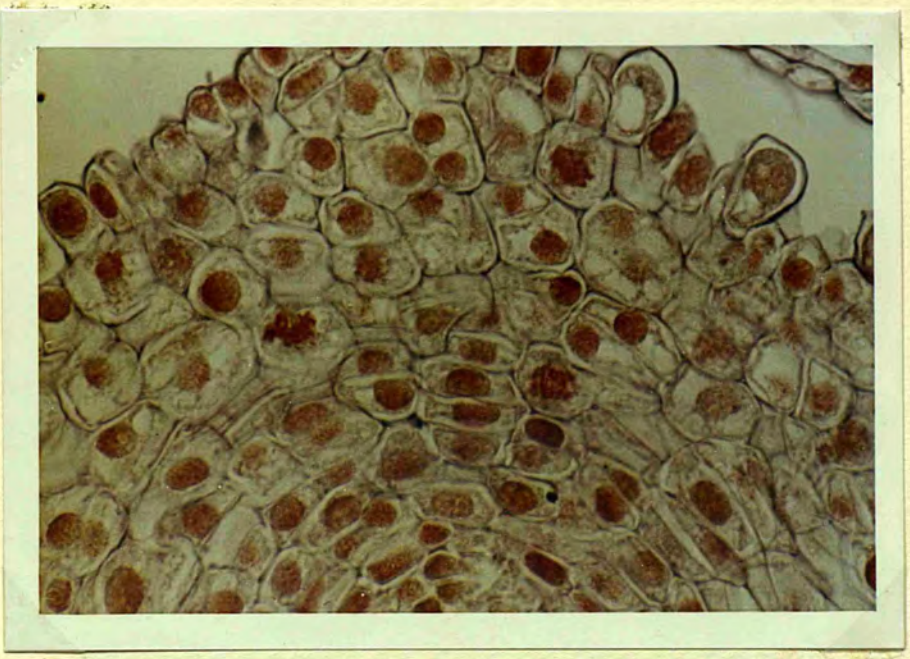
Pl.28



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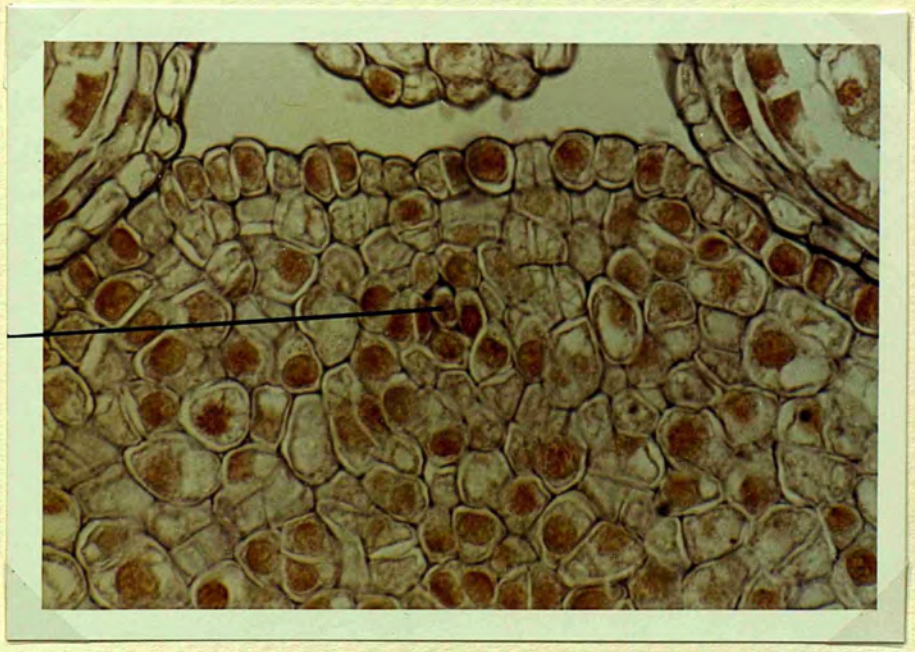

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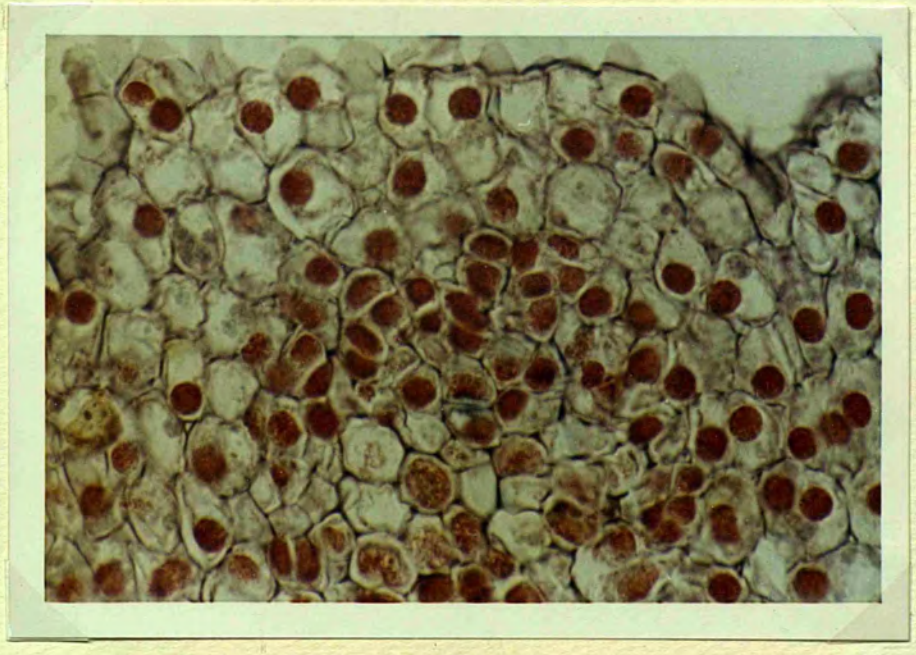
Pl. 29



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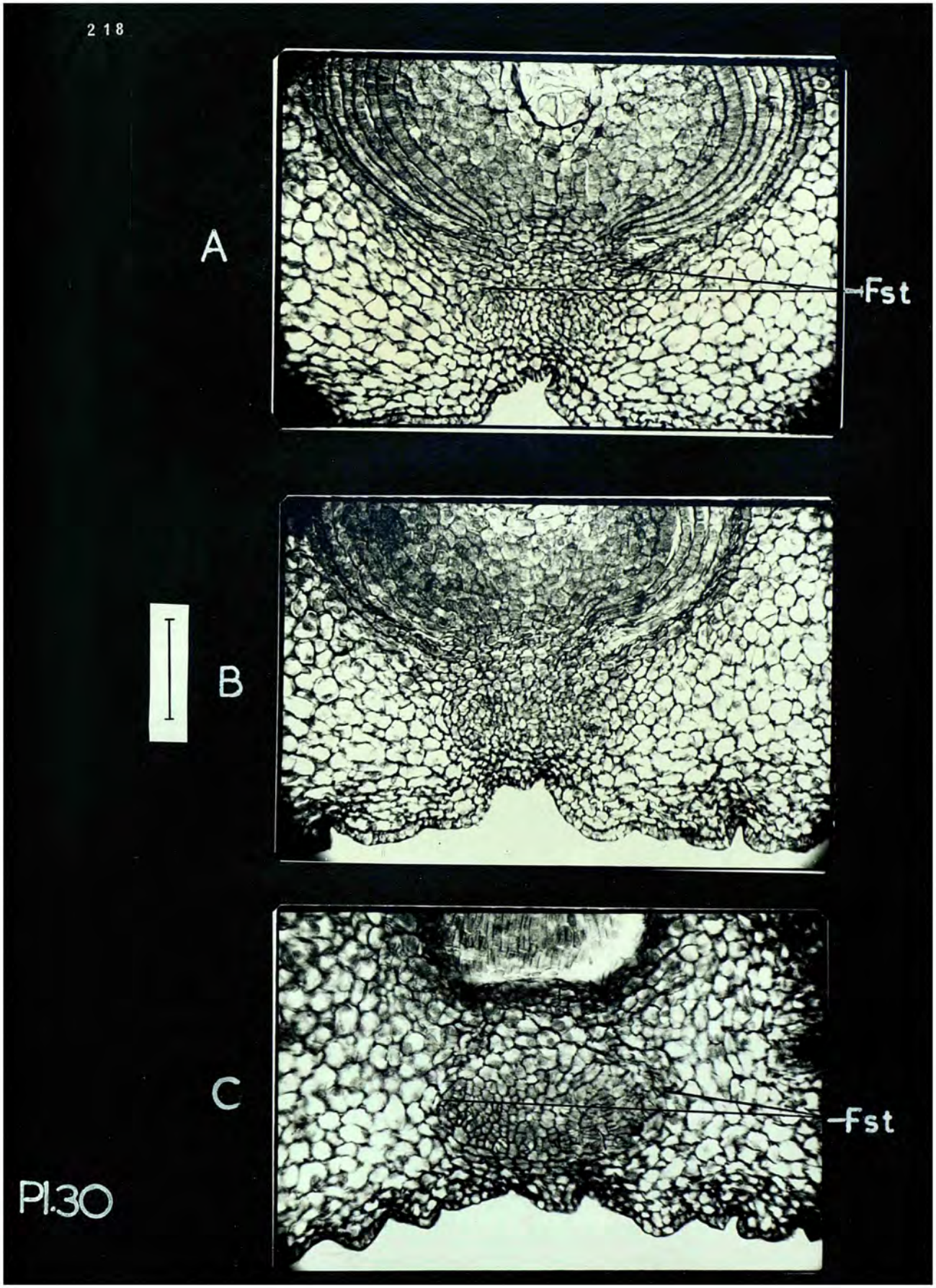
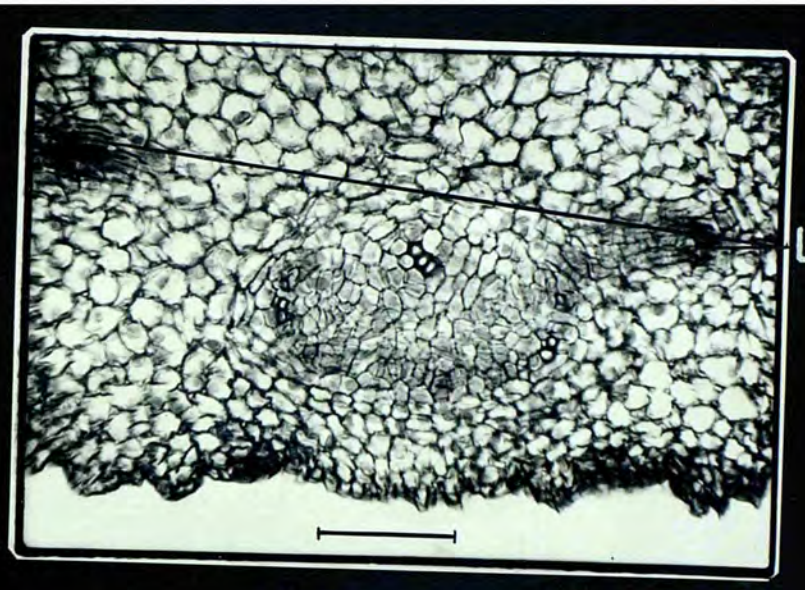
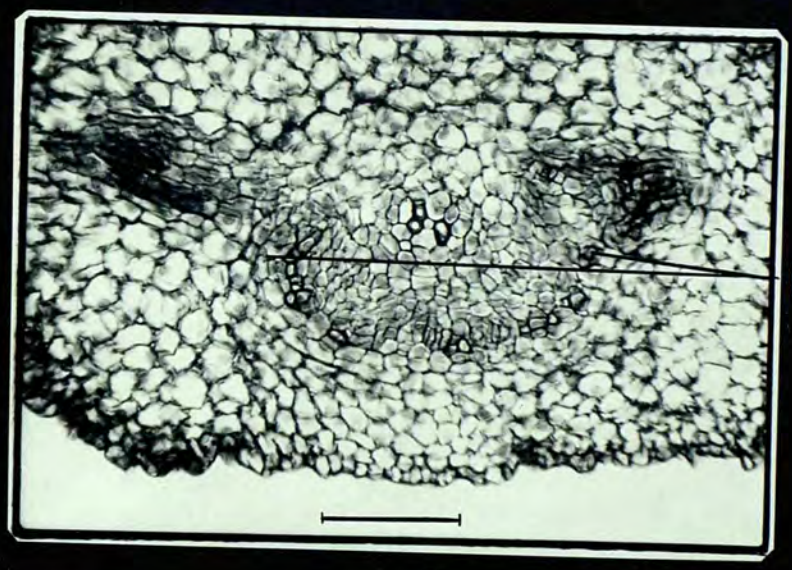
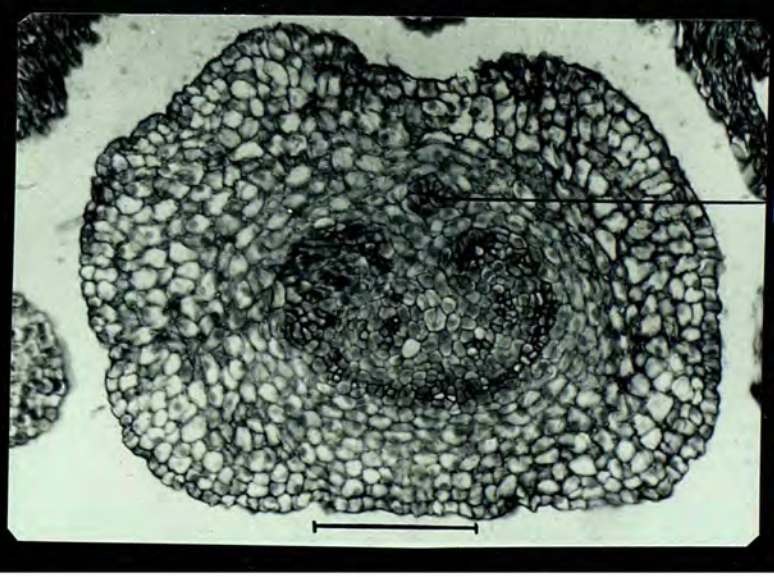
Pl.29



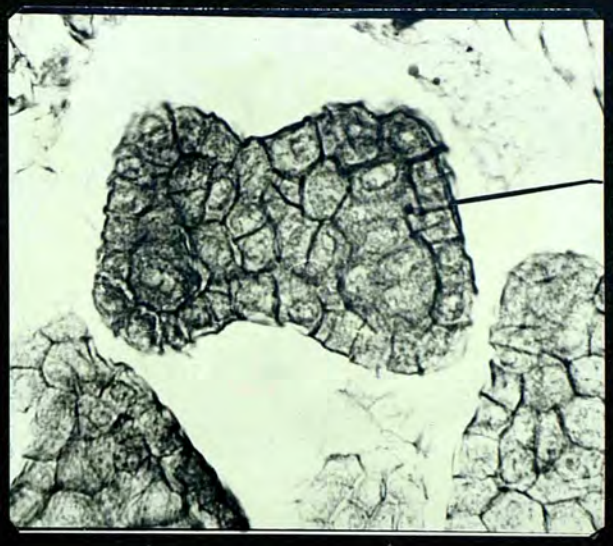
Pl.30







Pl.30



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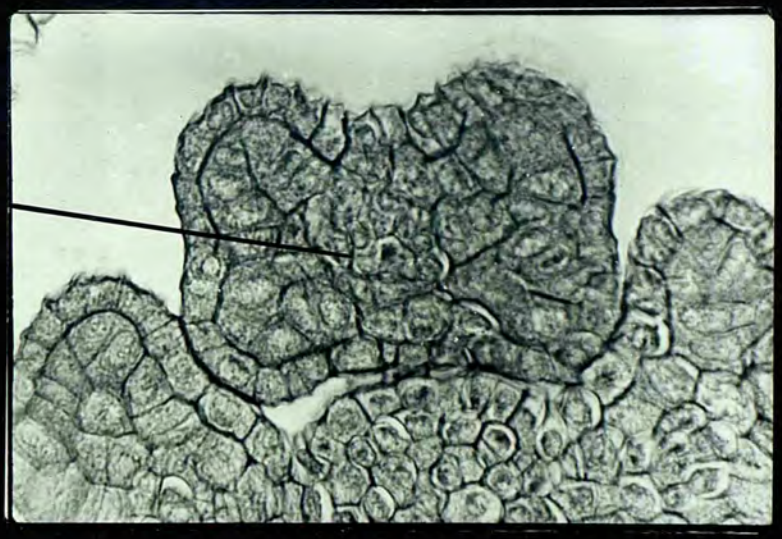
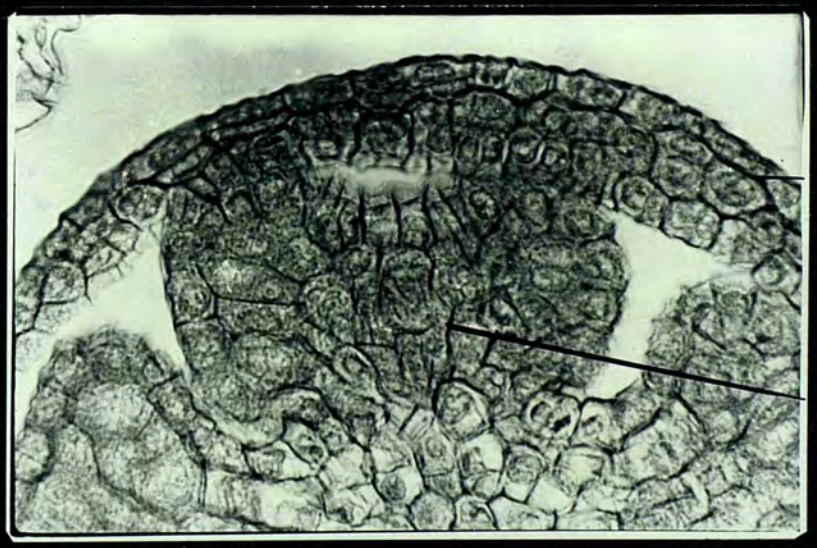
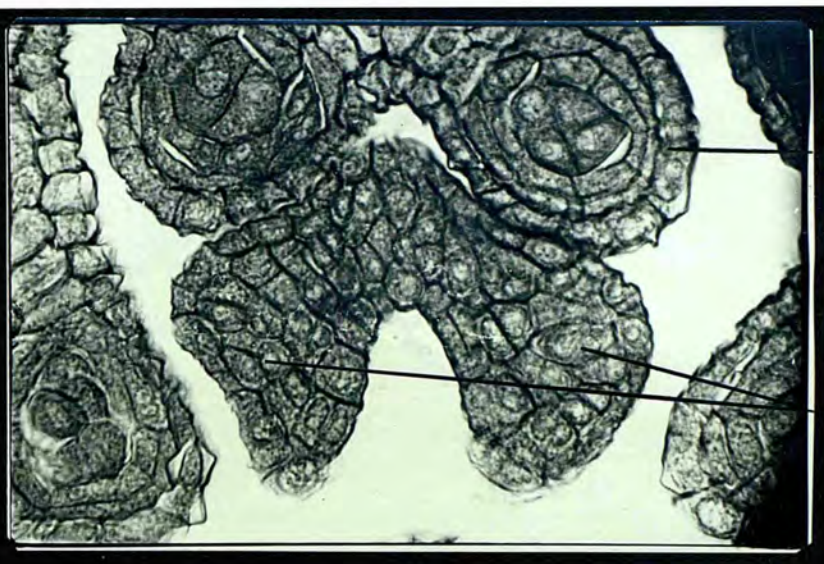




C

Pl.31



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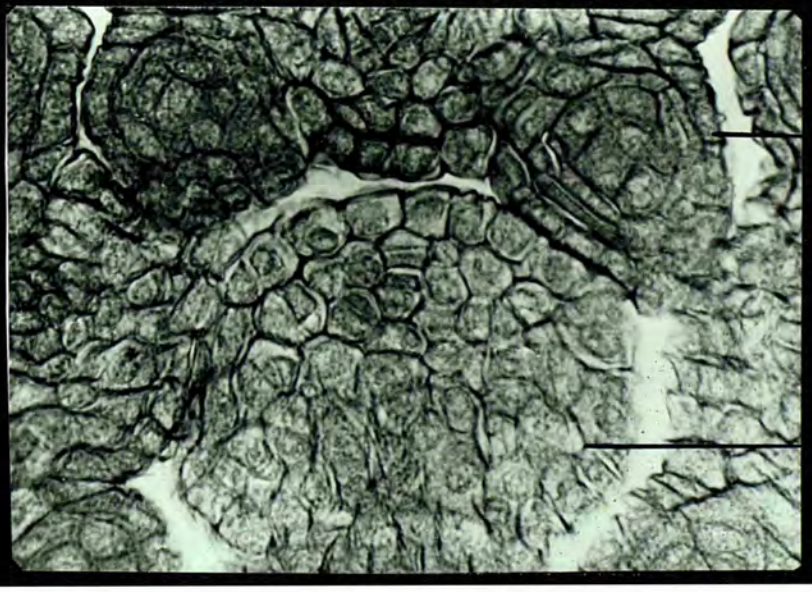

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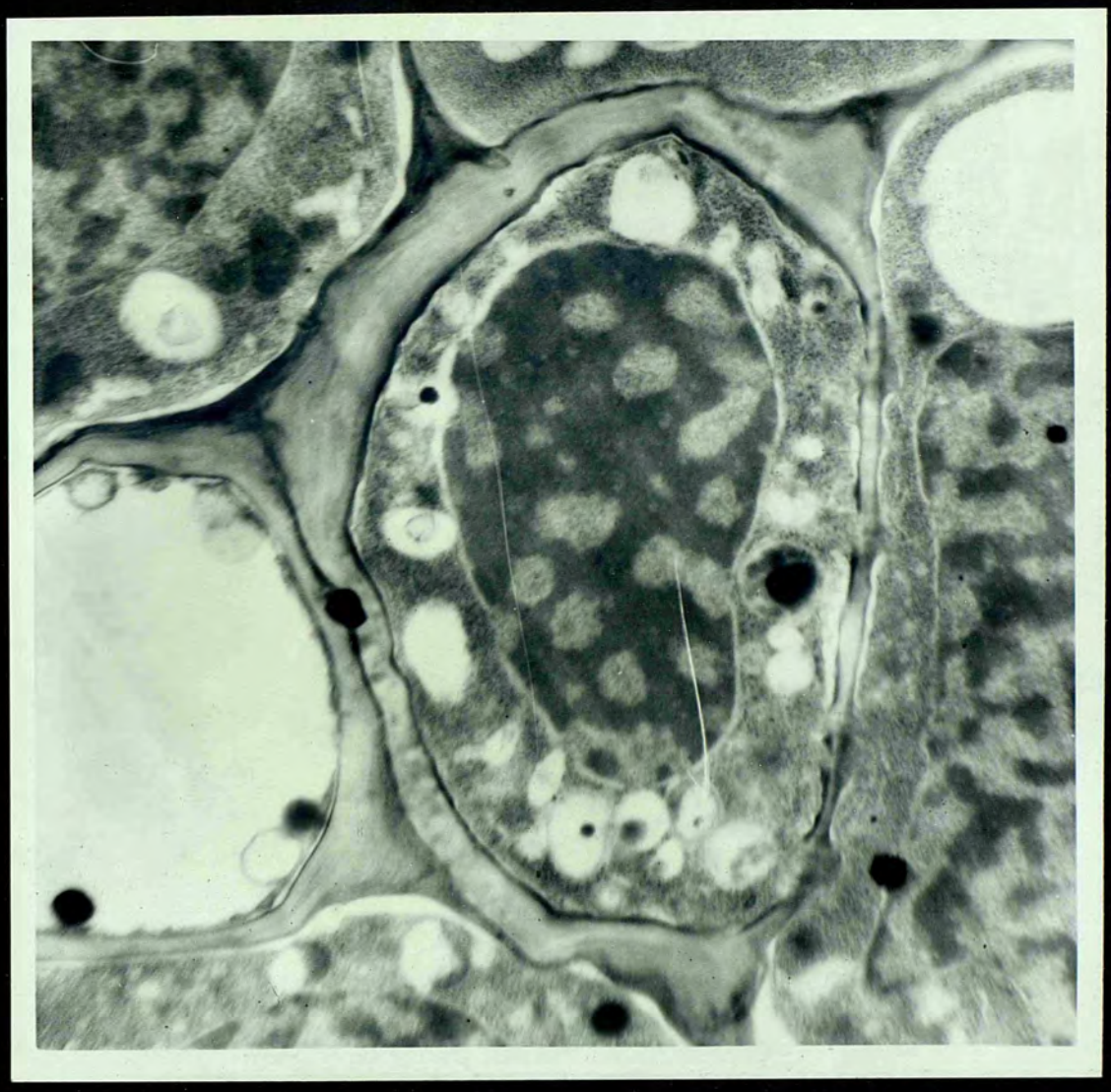
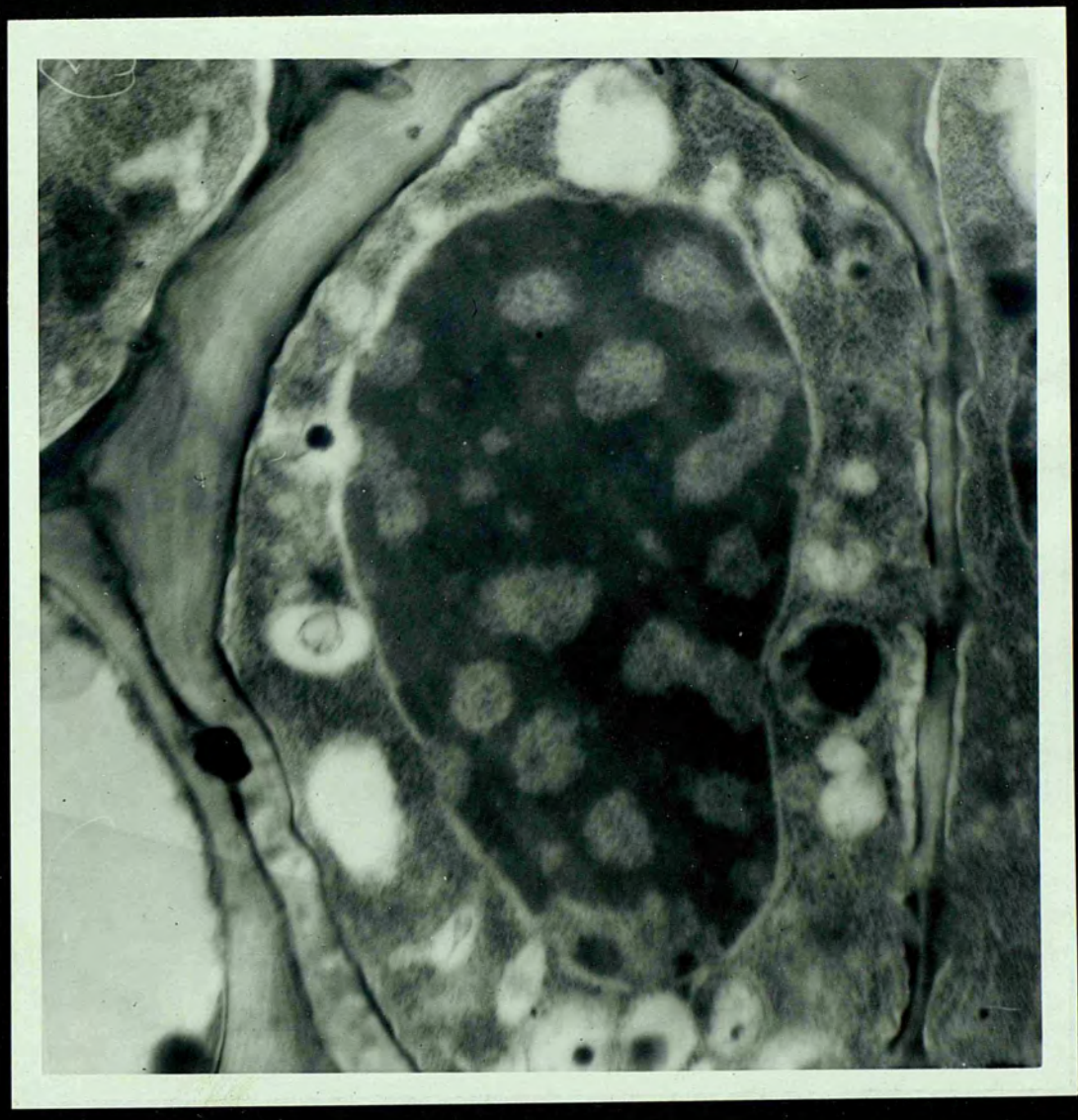
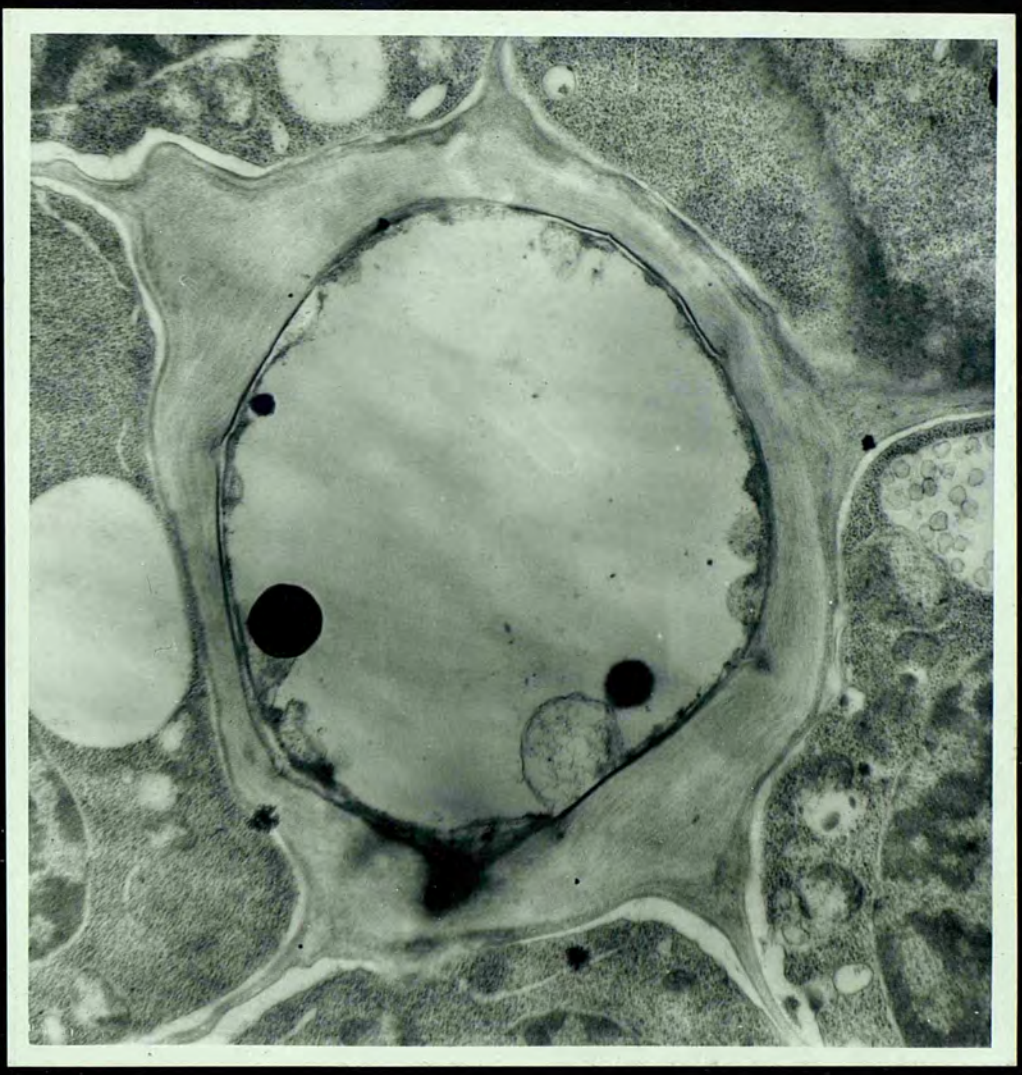
Pl.32



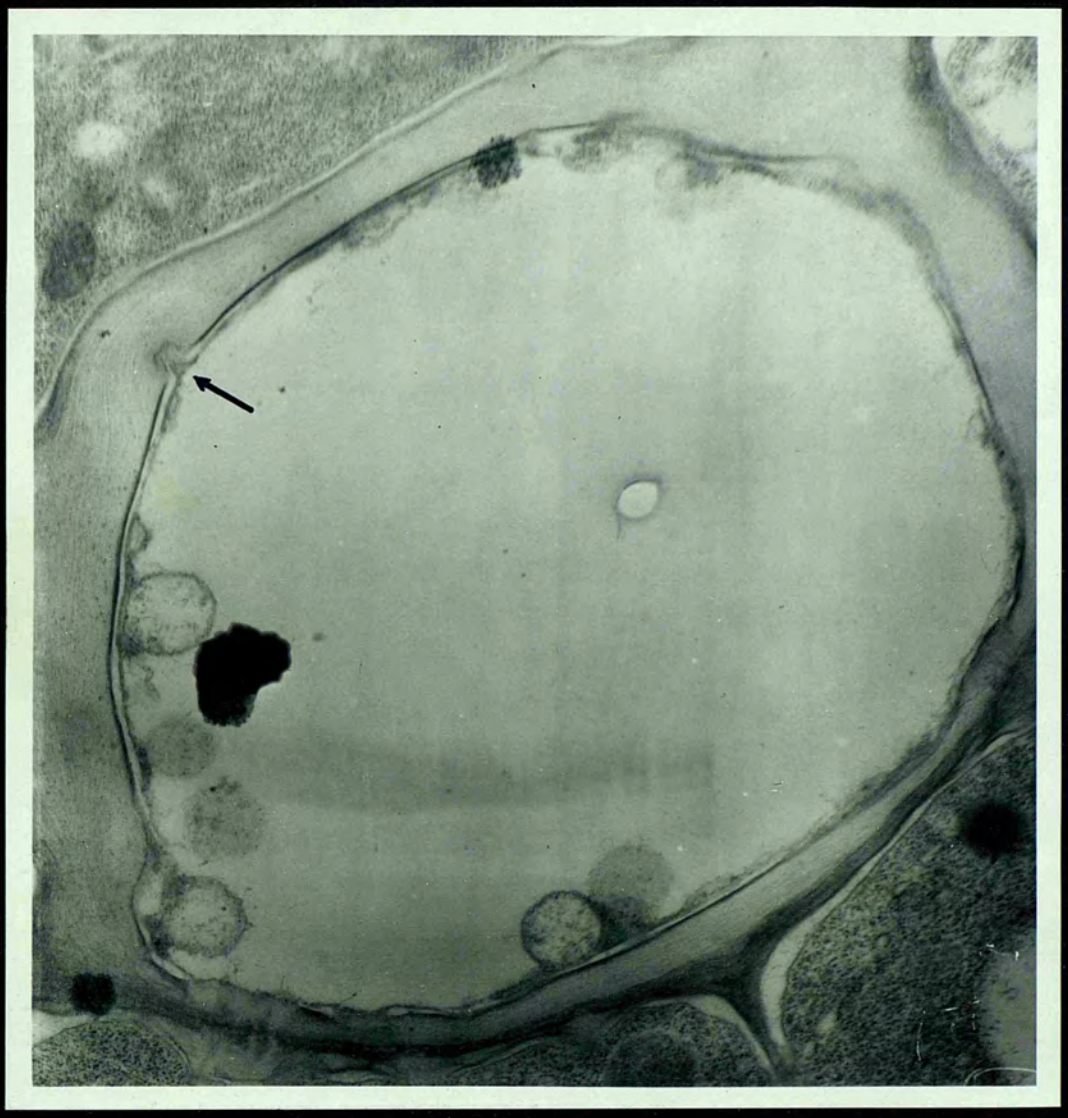
Pl. 33



Pl.34



Pl.35



Pl.36

Abbreviations used in the ILLUSTRATIONS.

AS = Anterior stamen.

Ast = Anterior stigma.

AW = Awn.

C = Carpel (C' represents anterior and C" posterior portion of carpel).

Ch = Carpellary hairs.

Con = Connective.

D = Dermatogen.

Dd = Dermatogen division.

Dp = Divisions for palea.

F = Filament.

Fpcst = Funicular procambial strand.

Fst = Funicular strand (bundle).

Fp = Floret primordium.

Fa = Floral axis.

Fp" = Anterio-posteriorly flattened floret primordium.

G = Glumes (G1 = first and G2 second glume).

Gp = Growing point.

H = Hypodermis.

Hd = Hypodermal division.

Ics = Intercellular spaces.

Int = Integuments (O. Int = outer integument I. Int = inner integument.

L = Lemma (L_1 to L_5 indicating the position of successive lemmas on spikelet axis).

Ls = Lateral stamen.

Le = Vegetative leaf.

Lst = Lateral strands.

Lo = Lodicules.

Lotr = Lodicular traces.

Lpcst = Lateral procambial strand.

Mme = Megaspore mother cell.

Mst = Median strand.

Mpcst = Median procambial strand.

Ov = Ovule.

P = Palea.

Pcst = Procambial strand.

Pp = Posterior protrusion.

Pph = Protophloem.

Pxy = Protoxylem.

S = Stamen.

Sh = Subhypodermis.

Stt = Stigmoid tissue.

St = Stigma.

Sth = Stigmatic hairs.

Stc = Styler canal.

Vc = Vascular cylinder.

Vst = Vascular strand.