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Develo̧pment of Mantle Leaves in *Platycerium bifurcatum* (Polypodiaceae) II. Vascular System

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Platycerium bifurcatum(Polypodiaceae) 잎의 발생 II.유관속계

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ABSTRACT

Procambium develops in the histogenetical zone below the marginal meristem. The development of procambium is correlated with the developmental stage of the leaf. As long as the marginal cells are crescent-shaped, the first-order procambium develops. When the marginal cell becomes wedge-shaped, the higher order procambium appears. The reticulate venation is developed through forking and anastomosis. The meshes of the first order enlarge in the process of leaf growth and the meshes of the second and third order develop in the meshes of the first-order through the differentiation of the residual meristem. Therefore, the venation is hierachically arranged and is as recognizable from the thickening of the veins. The outermost vein is produced parallel to the leaf margin, in which the differences between the costal and intercostal parts of the marginal meristem are removed. The endodermis and pericycle differentiate from the same mother cell. The procambium and sclerenchyma originate from a common source during the first developmental stage. A small cellular cluster lies within the parenchyma at the upper and lower sides of the procambial trace and differentiates into sclerenchyma.

INTRODUCTION

Leaf venation in ferns aroused the interest of early botanists as a result of its esthetic pattern. Hofmeister (1857) has studied the development of venation in several *Pteris* species. In a more detailed and fundamental study, Sadebeck (1873, 1874) described the development of leaf venation.

Goebel (1922) has taken the view that the marginal meristem is divided into costal and intercostal meristem and has determinable influence on the development of the venation.

Apart from this, studies of Hagmann (1964, 1965, 1967) have further illuminated the developmental process of leaf and leaf venation. He argues that the developmental process may be divided into morphogenesis and histogenesis. As a result, the procambium would not develop in the marginal meristem of very young leaflet, but follows its growth. Leist (1975) supports the study of Hagemann. Some researchers have concentrated on the use of venation in systematics (Rury and Dickison, 1977). Their consideration needs to be given not only to adult venation but also the developmental characteristics in systematics (Hetterscheid and Hennipman, 1984). This paper employs development of extraordinary venation of mantle leaves and contributes to make clearly development of reticulate venation in fern leaves.

MATERIALS AND METHODS

Platycerium bifurcatum plant specimens were collected at the University of Heidelberg Botanical Garden (HEID). Shoot apices with leaf primordia and young leaves at different stages of development were fixed in FPA and dehydrated in an ethanol-buthanol series. The material was embedded in paraplast and serial-sectioned at 5-10 μm. These sections were then mounted and stained in haematoxylin after Delafield.

For the preparation of clearings, small segments of leaf margin at different developmental stages were dehydrated in ethanol, cleared in methylbenzoat and mounted unstained in canada balsam. The leaf clearings were then photograped with Nomarski differential interference contrast optics.

RESULTS

Development of the procambium. The development of the procambium is a primary histogenesis in the histogenetical zone below the marginal meristem. The marginal meristem is produced by the leaf apical cell and consists of marginal initials and a submarginal meristem (Hagemann, 1965). Procambium develops very early in the leaf initiation and enters from the rhizome acropetal to the leaf. Many traces of procambium are arranged elliptically in the cross section of the very young leaf (Fig. 1). Oppositely lying traces meet together later and lie in one plain (Fig. 2). The transition can be better observed in a series of sections. If the procambial traces attain a length of three to four cells, they fork and anastomose with neighbouring branches. The cells of intercostal plain lying between the procambial traces can be distinguished from the procambium by their round nuclei and smaller size. All procambial traces in the young leaf fork and anastomose repeatedly. Consequently, a reticular venation develops. Procambium and parenchyma differentiate in the residual meristem through differently orientated divisions. The cubic cells of the residual meristem develop from the submarginal meristem through transversal divisions. The isodiametrical cells result from division in all directions of the leaf plain. The residual meristem lying near the leaf margin differentiates into procambium through multidirectional divisions. The first-order procambium develops acropetally for some distance (five to six cells) from the marginal cells. The development of the procambium continues with the initiation of higher-order venation, thus the youngest veins are always connected to older veins.

The differentiation of the veins is correlated with the developmental stages of the leaf. As long as the marginal cells are crescent-shaped, the first-order procambium can be found. In a transverse section at about 300 μm from the leaf base the traces of procambium have already forked twice (Fig. 3). In a late stage, about 500 μm from the leaf base, the procambial traces have forked three times and are stronger in the base than in the middle and near the leaf margin (Fig. 4). When the crescent-shaped marginal cells become wedge-shaped, the higher-order procambium appears in the base and middle of the leaf (Fig. 7,8).

The increase in leaf size is based on anticlinal division of marginal cells (Fig. 5). The marginal cells cease dividing once the leaf has reached about 2 cm in length. Leaf growth continues and the external mesh is produced parallel to the leaf margin once the leaf is 3 cm long (Fig. 6). This late growth of the leaf margin is not achieved through division of the marginal cells but through division of the submarginal meristem (Lee, 1989), thus removing the differences between costal and intercostal parts of the marginal meristem. The tangential extension of the marginal cells results in further growth of the leaf and the loss of its meristematic properties. In a transverse section of a leaf of about 800 μm in length, the main traces of the procambium have forked three times at right angles to the leaf margin (Fig. 7). The meshes of the first order enlarge during leaf growth. The meshes of the second and third order develop in the meshes of the first order. In a transverse section of a leaf about 1500 μm in length, the procambium traces are arranged hierachically in the base and middle of the leaf (Fig. 8), while near the leaf margin they are still oriented vertically to the leaf margin. When the mesh of the first order attains quantity of cells (30-40 cells in transversal section) through the divisions of the cells of the residual meristem, the mesh of second order develops within the first-order mesh and again the mesh of the third order within that of the second. Because the intensity of growth of the intercostal plain is evenly distributed, the initiation of veins is possible from all sides. This process begins next to one vein by the division of a cell of the intercostal plain and eventually connects the existing veins. The residual meristem within the mesh operates on the development of the procambium as a center of attraction and later participates in the extension of the leaf plain. Furthermore, many subsidiary meshes can be induced. Therefore, the reticulate venation is arranged hierachically as recognizable from the thickness of the veins.

The main veins of young leaves anastomose two or three times during longitudinal growth. This process ceases after they have forked two or three times because the periplastic growth in the marginal meristem continues uninterruptedly and results in a radial increase. The radial growth is stronger than growth in the lower part of the leaf. Here the subsidiary veins develop in the intercostal plain between the main veins. This process continues until adult size is reached. In the adult stage, the main meshes consist of main and subsidiary veins in the middle of the leaf and near the leaf margin, while they consist only of main veins in the base of the leaf.

The differentiation of the vascular bandle from the procambium. Differentiation of the vascular bundle from the procambium follows different patterns. The thickening of the components of the vascular bundle is not equally distributed or correlated with the thickening of the leaf. The different patterns of development of the vascular tissue can be observed in a cross section of the leaf of about 2,5 cm in length (Fig. 10-13). The differentiation of vascular tissue occurs partly within the procambial bundle, but also on that part of the procambium which divides further. In this section (Fig. 10), the protophloem (PP) is already differentiated at a distance of about 240 μm from the leaf margin even though the other vascular elements are not yet differentiated. The vascular bundle is separated from the mesophyll by a sheath. The vsccular bundle located about 700 μm away from the leaf margin has protophloem (PP) on its lower and protoxylem (PX) on its upper side (Fig. 11). The endodermis (E) and pericycle (P) are only partially differentiated from the same mother cell. Further differentiation tends toward a concentrical arrangement of the vascular tissue. Thus metaxylem (MX) is in the middle of the vascular bundle with the phloem surrounding it while the endodermis is fully differentiated (Fig. 12). This stage can be seen in the middle of the leaf. The difference in degree of differentiation of the vascular tissue is substantial between the base and the margin of the leaf. Therefore, at this stage the vascular tissue in the base is fully differentiated (Fig. 13). The thickening of the vascular bundle naturally decreases from the base of the leaf towards the margin.

Development of sclerenchyma. Sclerenchyma and parenchyma can be distinguished from each other at a very young stage. In a cross section of a young leaf, a small cluster of cells (CC) observed lying within the parenchyma at the upper and lower side of the procambial traces. This maintains meristematic properties (Fig. 9). This cellular cluster differentiates into sclerenchyma of the first order. This cluster and further sclerenchyma can not be distinguished from the procambium during the early developmental stage because both originate from the same source. Further sclerenchyma is separated from the procambium through the development of intermittent parenchyma. The vascular bundle of the second and third order differentiate from the residual meristem. The sclerenchyma differentiates on the upper and lower side of the vascular bundle. Consequently, the sclerenchyma and subsidiary veins are isolated from each other at the beginning of development.

DISCUSSION

Development of the vascular bundles. The development of the vascular bundles is connected with the marginal meristem which divides into a costal and an intercostal meristem (Goebel, 1922). Vein formation depends on the correlated function of the costal and intercostal meristem. Pray (1960), after the study of *Regnellidium,* has agreed with Goebel. Hagemann (1964), however, has suggested a new concept for leaf development of Pteridophytes after fundamental comparative studies of developmental processes, in which he argues that morphogenesis and histogenesis are different processes and that they take place one after the other. The shape is constructed in the promeristem and histogenetical differentiation follows morphogenesis, thus morphogenesis influences histogenesis.

The initiation of forked veins follows the process of primary morphogenesis and responds with formation of its own pattern if the fractination of the marginal meristem is discontinued. But half-meristematic zone under the marginal meristem is responsible for the formation of reticulate venation and for the whole leaf plain at the stage of vigorous growth. Hagemann (1965) has tried to show that the venation pattern in *Adiantum* depends on morphogenesis. He also tries to demonstrate how the differentiation of tissue in the marginal zone of the leaf is strongly influenced by the growth direction by showing that radially growing forked veins in the marginal zone of the leaf of *Regnellidium* become united by a marginal vein once dilation surpasses the radial increase (Hagemann, 1967). This result conforms the correlation between the progress of the procambium and the direction of main growth (Goebel, 1922, p. 1193). The study of the developmental process also shows that the histogenetical differentiation of the leaf plain does not start at the margin of the leaflet but from its base. The descendants of the young marginal meristem have no determinable influence on venation. As long as the radial growth is strongest, the development of the vascular bundles follows the direction of growth. Leist (1975) obtained similar results in *Stenochlaena tenuifolia.*

The procambium develops at some distance from the marginal meristem. The distance in young leaves is larger than in old leaves. As the center of attraction, the growing marginal meristem requires building materials. Each living cell and particularly embryonic cells can conduct material (Sachs, 1984), and the vascular bundle is the source for material attracted by the mrginal meristem. This is one of the reasons for the specialization of cells into vascular elements (Sachs, 1984). In *Adiantum,* the procambial traces follow the dilation of the growing marginal meristem and must widen in a fan-shaped pattern in accordance with the dilation. Once it exceeds a critical size, subdivision of procambium into two smaller traces takes place and parenchyma is formed by the residual meristem between them. This process is an example for pattern formation controlling the distance between traces.

Recently Sachs (1975, 1981, 1984) has studied the relation between hormone transport and the development of vascular bundles. Auxin from young leaves can influence the differentiation of the tissues. Growth is polar in form and the vascular bundle develops along the path of transport. After Sachs this is reflected in the proportional pattern of formation of the leaf plain. The process of pattern formation of reticulate vanation also is clearly polarized. From his numerous pictures of reticulate venation he concludes that the development of pattern is not caused by the procambium but by the homogeneous residual meristem in the intercostal plain. This process is connected with the formation of intercellular spaces. As long as the growth of the marginal meristem is dominate, the polar differentiation of the main vein is clear. The polar differentiation of subsidiary veins in a reticulate pattern, however, is lost through the multidirectional growth of the leaf plain.

The hierachical arrangement of reticulate venation in *Platycerium bifurcatum* results from different developmental processes. The main veins fork at certain distance and in agreement with the increasing dilation of the leaf margin. Parallel running veins often unite with each other or through commissural veins at unequal distance, so that meshes are formed. Goebel (1922) studied a comparable process of mesh building in *Anemia phyllitidis.* The vein in the leaflets of *Anemia phyllitidis* develops a partially closed venation towards the leaflet margin through two methods of union and commissure formation. The union of veins in longitudinally elongated leaves gives the impression that length growth partially surpasses width growth. But the union of the neighbouring forks takes place more rarely than the forking, so that the main veins multiply in relation to the growth of the leaf plain. The closed venation of *Acrostichm aureum* (Leist, 1976) develops differently. The side veins differentiate from the middle vein and grow towards the leaf margin. The veins follow the direction of longitudinal growth of the pinna at the early stage because the marginal meristem grows rapidly by anticlinal directions. The veins are deflected towards the leaf tip and differentiate until contact to the marginal veins, thus a mesh is formed. The mesh is oval and elongated near the leaf tip. New meshes are produced towards the margin as growth of the leaf plain continues. Three anticlinal veins lie on one periclinal vein. In this way, a regular reticulate venation is produced. The intercostal plains differentiate in the growing leaf plain and are surrounded by the reticulate vascular bundles. The reticulate venations of *Anemia phyllitidis* and *Achrostichum aureum* are not hierachical.

The radially forked main veins in the internal part of a mantle leaf of *Platycerium bifurcatum* are polar in structure but become more and more apolar in structure towards the leaf margin. The subsidiary meshes are limited by polar veins on one side and newly developing apolar veins on the other. Therefore, the mantle leaf of *Platycerium bifurcatum* has a hierachically arranged reticulate venation. The subsidiary veins differentiate independently and at a long distance from the leaf margin. The differentiation of reticulate venation supplies conclusive arguments against the division of the marginal meristem into a costal and an intercostal section.

Sclerenchyma. The sclerenchyma in the ferns can be found in the ground tissue, where, above all, it surrounds the vascular bundles. The sclerenchyma bundles in the cortex and pith, so-called pseudoveins, are well-known in many fern leaves (Helm, 1935; Bower II, 1923, p. 237). The sclerenchyma bundles in mantle leaves are related to the vascular bundles and follow them at a certain distance from their upper and lower sides. This contrasts with many other ferns, therefore, it is questionable whether the sclerenchyma bundles can be treated as parts of the vascular bundles. The vascular bundles of many ferns are sheathed by sclerenchyma on the outside of the endodermis. Many plant anatomists consider the endodermis to be part of the cortex, particularly in the case of root (Esau, 1969, p. 357). As suggested by Hagemann (personal communication) the sclerenchyma may be a component of the ground tissue when the endodermis is observed close to the vascular tissue, and later may belong functionally to the vascular bundles. A great number of ferns have their sclerenchyma further away from the vascular bundles and it is seperated by parenchyma from the endodermis. The band-shaped sclerenchyma bundles in the rhizome of *Pteridium aquilinum* lie between the band-shaped vascular bundles and their cells are elongated in the longitudinal direction. Therefore, it can be expected the sclerenchyma bundles are not distinguishable from the vascular bundles at their procambial stage.

The sclerenchyma bundles of *Platycerium bifurtum* lie in the same plain on the upper and lower sides of the vascular bundle and follow the vascular bundles. This suggests that the sclerenchyma develop from the residual meristem together with the vascular bundles. The parenchyma and sclerenchyma bundles develop from portion of the residual meristems lying above and below the vascular bundles when the thick vascular bundles develop in the middle of the residual meristem. But if the thin vascular bundles develop from the middle of the residual meristem, a large part of the residual meristem is maintained and most of it differentiates into parenchyma. Therefore, the sclerenchyma bundles which develop from the upper and lower sides of residual meristem move further away from the thinner vascular bundles and lie in the same plain with the primary sclerenchyma bundles. It can, therefore, be postulated that the thickening of the residual meristem is connected with the separation of the vascular and sclerenchyma bundles.

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적 요

전형성층은 주변분열조직 하부의 조직분화 지역에서 발생하며, 잎의 발생과 연관되어 있다. 주변분열세포가 초승달모양일 때 주맥의 전형성층이 발생하고 쐐기모양으로 변한 다음에 세맥의 전형성층이 나타난다. 망상엽맥계는 분지와 융합에 의해 이루어진다. 주맥으로 이루어진 1차망은 잎의 생장과 더불어 커지고 2, 3차망은 잔여분열조질의 활동으로 1차망 안에 발생한다. 그러므로 엽맥은 계급적으로 배열되고 엽맥의 굵기에 의해 구별된다. 최외곽 엽맥은 잎의 주변부와 병행으로 발생하면서 주변분열조질의 costal 과 intercostal 부분의 구별이 없어진다. 내피와 내초는 동일세포로부터 분화되고 전형성층과 후막조직도 동일근원으로부터 기원된다. 전형성층엽맥 위 아래쪽에 위치한 유조직속에 존재하는 작은 세포로 이루어진 세포군이 후막조직으로 분화한다.

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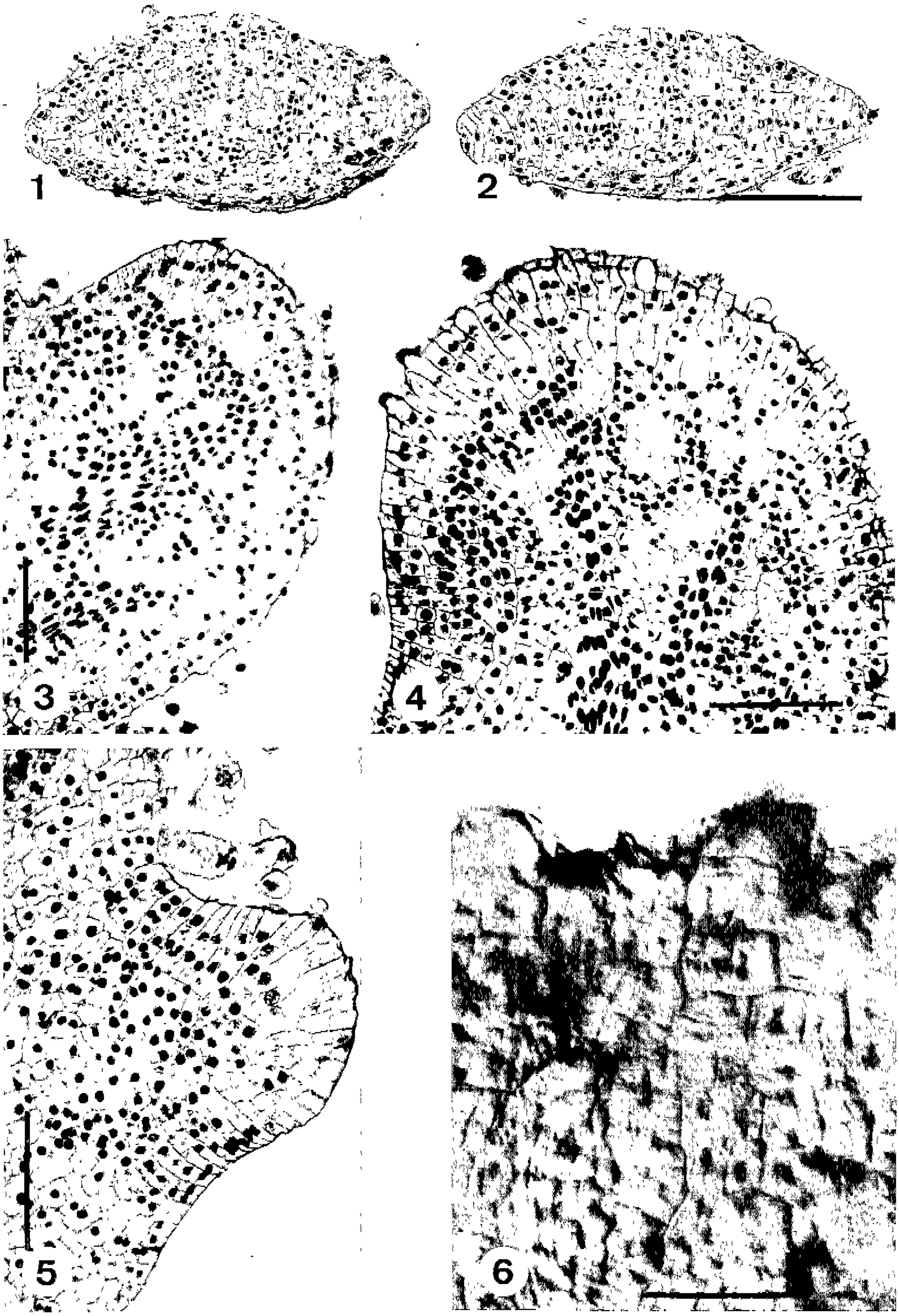
Explanation of Figures

Fig. 1-6. Cross section at base of a young lesf. Many traces of procambium are arranged elliptically. Bar: 100 μm. 2. Cross section at an above part of the same leaf. The oppositely lying traces lie in a plain. Bar: 100 μm. 3. Transversal section of leaf primordium at the length of ca. 300 μm. The traces of procambium have forked already twice. Bar: 100 μm. 4. Transversal section of leaf primordium at the length of ca. 500 μm, Bar: 100μm. 5. Transversal section of leaf primordium at the length of ca. 180 μm, Bar: 100 um. 6. Optic transversal section of leaf at the length of ca. 3 cm. Outermost meshes are produced parallel to the leaf margin. Bar: 50 μm.

Fig. 7-9. Transversal section of leaf primordium at the length of ca. 800 μm. The main traces of the procambium have forked three times at right angles to the leaf margin. Bar: 100 μm. 8. Transversal section of leaf primordium at the length of ca. 1500 μm. The procambium traces are arranged hierachically. Bar: 100 μm. 9. Cross section of the young leaf. Bar: 100 μm. CC: Cluster of cells.

Figs. 10-13. Cross sections of the vascular bundles at the length of ca. 2.5 cm, Bar: 100 μm. 10. Protophloem (PP) is differentiated at about 240 μm distance from the leaf margin. PP: Protophloem. 11. Protophloem (PP) on lower and protoxylem (PX) on upper side of the vascular bundle are differentiated at about 700 μm away from the leaf margin. PP: Protophloem, PX: Protoxylem, P: Pericycle. 12. Metaxylem (MX) and endodermis (E) are differentiated. MX: Metaxylem, E: Endodermis. 13. The vascular tissue in the leaf base is fully differentiated.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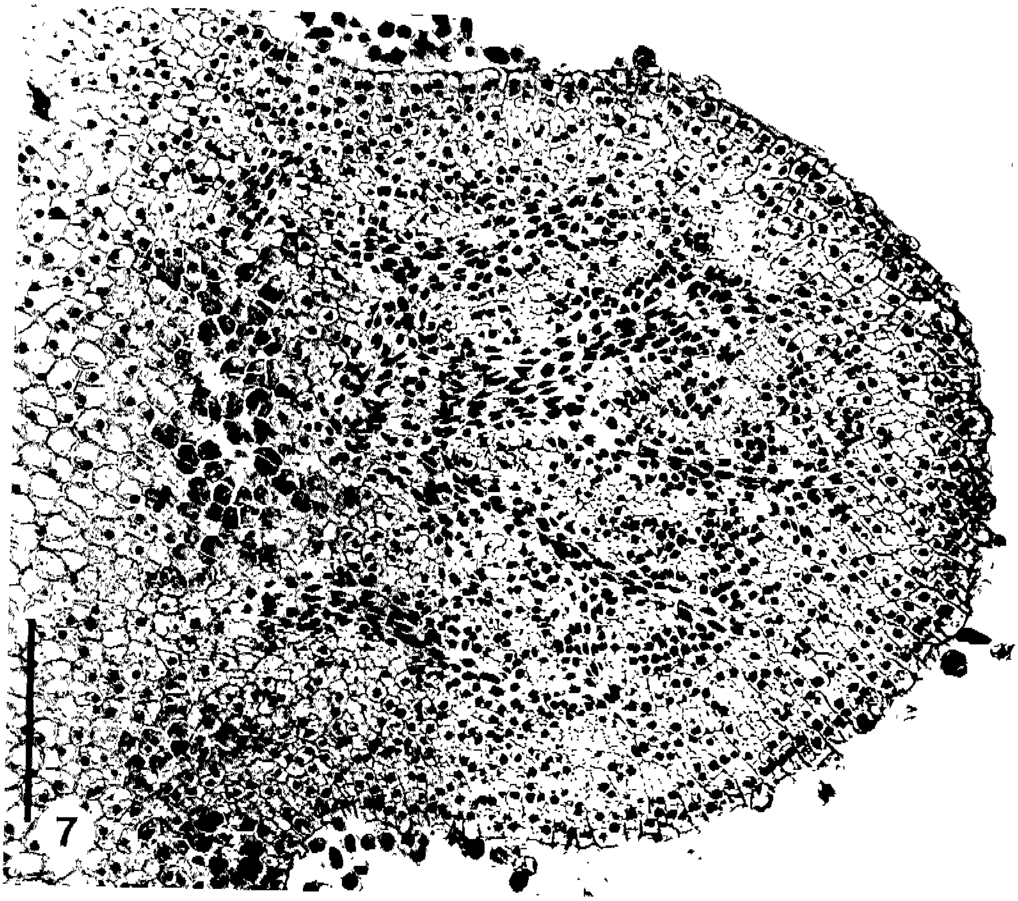
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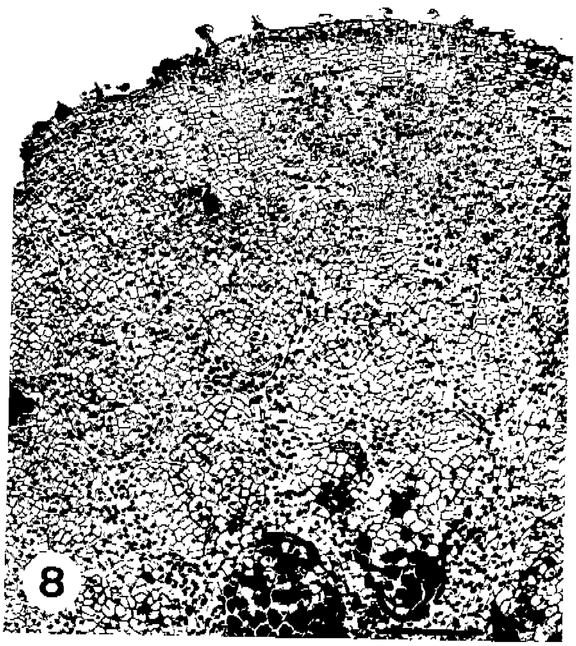
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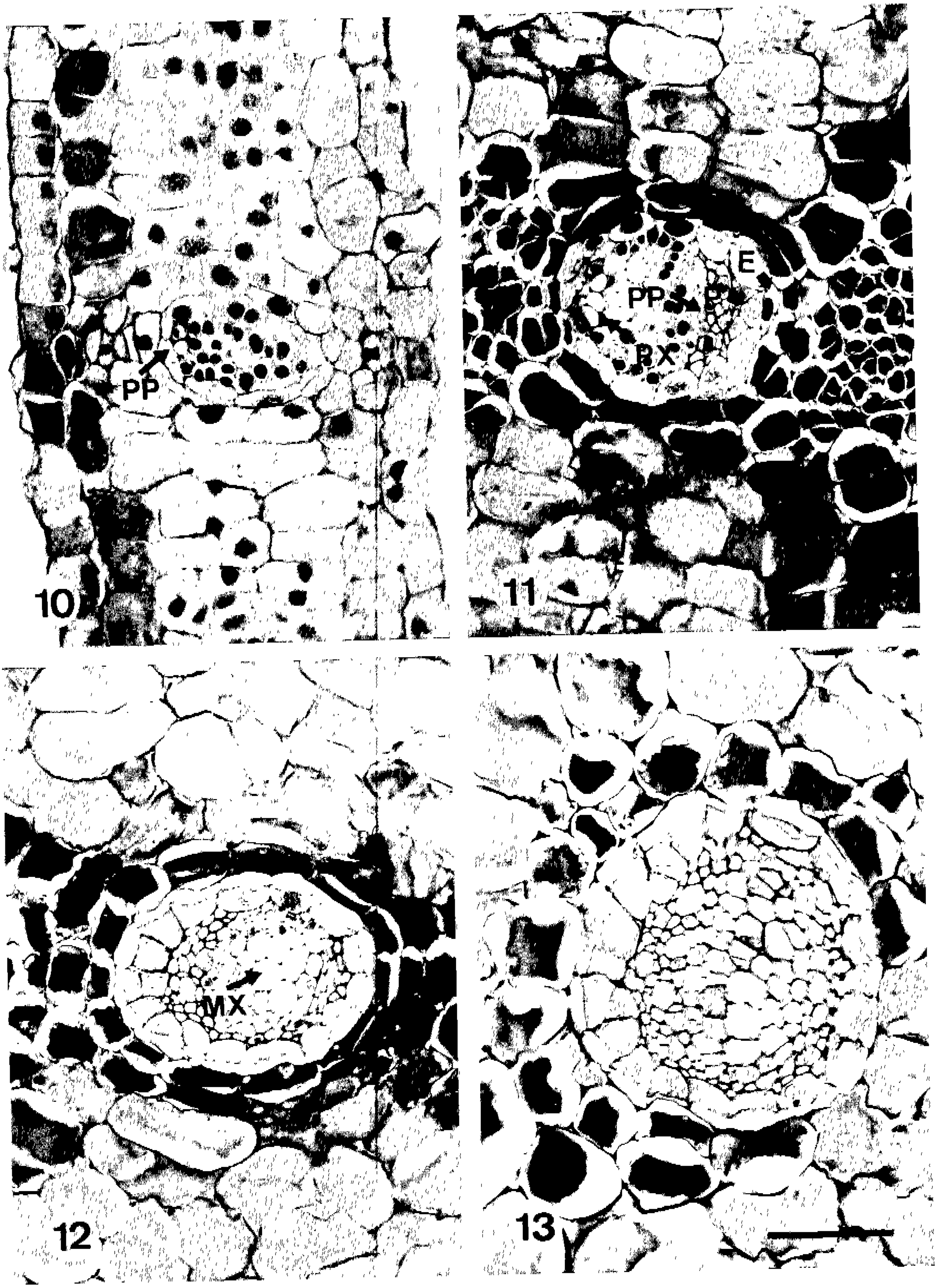
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