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MORPHOLOGY AND ANATOMY OF THE LEAF AND STOLON OF BUFFALO GRASS, BUCHLOE DACTYLOIDES (NUTT.) ENGELM.

A DISSERTATION

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MORPHOLOGY AND ANATOMY OF THE LEAF AND STOLON OF BUFFALO GRASS, BUCHLOE DACTYLOIDES (NUTT.) ENGELM.

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CHAPTER I

INTRODUCTION

Numerous anatomical investigations of the grasses have been reported. Bonnett (1959) made a thorough analysis of both vegetative and reproductive parts of the oat plant. The shoot apexes of a number of species, as an indication of their systematic relationship, were studied by Brown et al. (1957). Leaf blade anatomy was also studied by Brown (1958) for a similar purpose. Studies of root anatomy have been conducted by Clowes (1959) and Goodwin and Avers (1956). Inflorescence and floral anatomy have been described by Barnard (1955). Vascularization of the young wheat leaf has been reported by Sharman and Hitch (1967) and of the shoot of Zea mays by Esau (1943).

Buffalo grass has aroused the interest of many botanical investigators since its description as a new dioecious species by Engelmann (1858). Its dioecious nature was investigated by Plank (1892) and Schaffner (1920). A study of the life history of buffalo grass was conducted by Webb (1941). Its importance as a range species in the short grass plains in the western United States has brought it to the attention of agronomists (Wenger, 1943: Beetle, 1950) and ecologists (Albertson, 1946).

Although it has been investigated from these several standpoints,

no anatomical study has been conducted except the brief work of Metcalf (1960) and Arber (1934). As a valuable range species and a representative of the Chlorideae a detailed anatomical analysis of the leaves and stolons seems in order.

CHAPTER II

MATERIALS AND METHODS

Buffalo grass used in this investigation was collected on the University of Oklahoma North Campus at Norman. Leaf and stolon material for sectioning and staining was killed and fixed in formalin - acetic acid - 70% ethanol (Johansen, 1940). The fixed material was dehydrated in tertiary butyl-ethyl alcohol (Johansen, 1940). Shoots to be sectioned for study of the meristem were killed and fixed in Craf V (Sass, 1958). Shoots were then soaked 72 hours in a 25% solution of hydrofluoric acid. Dehydration of shoots was by the dioxane method (Sass, 1958). Shoots, leaves and stolons were embedded in paraffin (Paraplast, 56-57° C). Structures were sectioned at 8 or 10 microns. The best staining results were obtained by a modified tannic acid-iron alum method (Sharman, 1943). Studies of the epidermis were done on fresh material prepared and treated by the method of Metcalf (1960). Staining of the epidermis with an aqueous solution of safranin (Sharman, 1943) gave the best results.

CHAPTER III

DESCRIPTION

Morphology of the Leaf

Buffalo grass leaves are typical in appearance and form of those of other members of the family although anatomically they are in many ways distinctive. There is wide variation in length at maturity depending upon the position at which they occur on the plant. Mature leaves of tillers may be 20-30 cm long of which one sixth of the length is represented by the sheath. Leaves from stolons may be 5 cm long, of which 2 cm is represented by the sheath. In width the leaves are less variable. Two mm is the usual width of those used in this study, although leaves of 3 mm are reported in the literature (Beetle, 1950). The margins of the longer tiller leaves appear parallel as is characteristic of the grass leaf though in fact the margins do converge toward the distal end giving the leaf a gradually tapered tip. Blades of smaller leaves from the distal end of the stolon have more abruptly tapering margins than do longer tiller leaves.

While the stolon leaf blade may be as wide at the sheath collar as that of the tiller leaf blade, its total length may be only 2-3 cm compared to 20 cm for the latter. This combination of wide base and shorter length gives stolon leaf blades a more wedge-shaped appearance than that of the tiller leaf blades. In addition, the blades of stolon leaves attach to the sheath at an angle of 45 to 90 degrees with the long axis

of the culm. In some of the smaller distal leaves of the stolon the angle may be greater than 90 degrees so that the blade is actually reflexed with respect to the long axis of the culm and clasping sheath.

At the distal end of the stolon, imbricate leaf sheaths clasp the apical meristem. Here, the distichous arrangement of the leaves is especially evident. Elongation of internode and sheath appears to coincide during the initial period of growth. Later, growth of the leaf sheath ceases, while internodal elongation continues. This growth pattern produces the lengthened internodes of the stolons. At the proximal end of each internode the clasping sheath of the subtending leaf persists. In contrast with other leaves of the plant in which the length of the blade exceeds that of the sheath, sheaths of stolon leaves may be as long or longer than the blade.

The ligule consists of a single row of simple, straight, unbranched hairs attached at the collar where the sheath and blade are joined. The ligule lies parallel to the sheath and thus has the appearance of a ragged extension of its adaxial epidermis. It extends to both margins of the sheath, gradually diminishing in length toward the edge.

The mature blade, whether from a tiller or stolon, appears flat and pubescent to the unaided eye. Except where the blade narrows and rolls at the junction with the sheath, it is difficult to distinguish the upper (adaxial) from the lower (abaxial) surface. Nerves are not visible without the aid of a lens. The midrib is obscurely visible to the unaided eye.

Likewise without magnification, the abaxial surface of the sheath appears nerveless and glabrous.

Appearance of the Leaf with the Dissecting Microscope

Under the low magnification of a dissecting microscope many details of the leaf surfaces become apparent. The typical parallel venation of the grass family is clearly visible. The midvein and four to six large lateral veins traverse the length of the blade. On the adaxial surface the same veins appear as a series of parallel ridges running from the collar to the apex. In some leaves large lateral veins are more or less equidistant, each one being separated from its neighbor by four to six smaller veins: in others, more variation occurs.

Definitions of dermal appendages used throughout the paper follows Metcalf (1960). 1. Macrohairs: visible to naked eye or with hand-lens, often with a prominent base, straight, stiff, unicellular. 2. Microhairs: not visible to naked eye or with hand-lens, bicellular. 3. Pricklehairs: robust, sharp-pointed, cellular emergences, marginal only, visible with hand-lens.

Both surfaces of the blade are adorned with macrohairs. These are straight, unicellular, and unbranched, about 1 mm long, colorless, with acuminate tips (Fig. 12). In young, fresh material they are appressed and point toward the apex of the leaf. Some leaves have a profusion of macrohairs on the margins of the blade where they merge into the collar; others are devoid of hairs at this region, or are only sparsely hairy. When hairs do occur in this region of the leaf, they may also extend irregularly along the margin of the blade for a short distance away from the collar. Unlike the hairs on the surfaces of the leaf blade or sheath, these marginal hairs have prominent wart-like bases. Commonly only the wart-like base occurs, suggesting that the prominent base is a vestige or rudiment

of the absent hair.

In addition to macrohairs the margins of the blade also bear pricklehairs. These lie close to one another like sawteeth and point toward the apex(Fig. 12, 19).

The surfaces of the sheath resemble those of the blade. Ridges appear on the abaxial surface although the adaxial surface is smooth. Macrohairs are sparse on the abaxial surface except at the collar where they may be numerous and prominent. They are entirely absent from the adaxial surface except for the ligulate hairs at the collar.

The margins of the sheath are thin, colorless, and transparent for a distance of 0.5 mm from the edge. Pricklehairs are absent from the margins of the sheath.

The Apical Meristem of the Shoot

The meristem of the vegetative shoot in buffalo grass is small and dome-shaped (Fig. 6). It bears one or two leaf primordia and occasionally the initial of a third leaf. This characteristic places it among the grasses possessing a short-type apex as described by Sharman (1947).

A median longitudinal section through the plane of the leaves reveals the distichous arrangement that is characteristic of the family (Fig. 6). Leaf number one is represented by a periclinal division in the mantle. Directly opposite leaf number one is the primordium of leaf number two which has advanced in length to a position nearly equal to the apex of the meristem. The blade of leaf number three overtops the meristem apex while the initial of its sheath appears as an acutely angled appendage subtending the base of the meristem below the primordium of leaf number two.

Cytohistological zonation as defined and described by Popham (1951) for the angiosperm meristem can in part be recognized. One mantle layer covering the top of the meristem is clearly defined and a second less distinct layer may be present. Immediately below the apex, there is often an irregularly shaped subapical initial below which are two or more parallel rows of cells whose long axes lie parallel to the long axis of the meristem. These initials represent the central meristem. No clear distinction can be made between the cells constituting a central and a peripheral meristem. Popham noted in his discussion of angiosperm meristem types that those of the small members of the Gramineae often do not manifest the clear zonation found in other members of the family.

The Young Leaf Primordia

The median procambial bundle in the primordium of leaf number one is first discernible at a level near the tip of the meristem (Fig. 1, 7). It is persistent through four internodes (Fig. 2, 3 4, 5) and at the fifth node lies adjacent to the sclerenchyma cylinder surrounding the pith (Fig. 5). Throughout the course of the four youngest nodes (the four terminal unelongated internodes) there is no recognizable differentiation in the median vascular bundle other than the formation of procambium. Passing through the third (Fig. 3) and fourth (Fig. 4) nodes the strand diverges obliquely across the stolon but becomes vertically oriented again at the fifth node (Fig. 5). In the fifth node this vascular bundle is surrounded by a fiber sheath. It is adjacent to the pith and to a parenchymatous gap in the sclerenchyma cylinder.

Leaf number two has a well-differentiated median bundle (Fig. 1, 7)

and one pair of clearly discernible lateral procambial bundles at the level of the meristem apex. The median bundle is persistent through the next four older nodes (Fig. 2, 3 4, 5) and at the fifth node (Fig. 5) is adjacent to the sclerenchyma cylinder on the opposite side of the stem from that of the median bundle from the first leaf. It is similar in appearance to the first median bundle described above. Thus the vascularization of the youngest leaves is continuous with that of the older part of the stolon.

Leaf number three (Fig. 1 8, 9) at the level of the meristem apex possesses a median procambial bundle, a pair of major lateral procambial bundles and two pairs of minor procambial bundles. The median bundle has a bundle sheath, protoxylem and protophloem and what resembles a fascicular cambium. The major lateral bundles are similar to the median . . (Fig. 9). The minor bundles consist only of procambium similar to the lateral procambial bundles of the leaf number two (Fig. 7).

In the plane of the meristem apex, leaf number four (Fig. 1, 9, 10) has a prominent median procambial bundle (Fig. 1, 10), a well-differentiated pair of major lateral procambial bundles (Fig. 1, 9) and three pairs of minor bundles. Protoxylem and protophloem are present in the median bundles (Fig. 10) and in the lateral pair (Fig. 9). Two minor pairs consist only of procambium similar to lateral procambial bundles of leaf number two. A sheath surrounds the median bundle and the major lateral procambial bundle.

At the level of the meristem apex the distinction between blade and sheath has not developed in the first two (youngest) leaves. Slight invaginations (Fig. 7) on the adaxial side between veins on leaf number

two suggest the initiation of bulliform areas of the blade. The youngest leaf shows no comparable development. Leaves number three and four are sectioned in the region of the sheath as is indicated by the large-celled parenchymatous tissue and attenuated margins characteristic of the mature sheath. Radiate chlorenchyma and bulliform tissue are absent in all four of the youngest leaves. The margins of leaf number four (Fig. 9) are gradually tapered to a double layer of epidermis as is characteristic of the sheath. The margins of leaves number one and two are more abruptly tapered resembling those of a mature blade. Each of the four youngest leaves has a clearly defined epidermal layer but all four lack sclerification in the epidermal and subepidermal layers. One or two bundle sheaths are visible in the median bundles of the four youngest leaves. Major lateral bundles in the third and fourth leaves also possess well-marked bundle sheaths. At the stage of development represented by this section, these bundle sheaths are not differentiated as mestome or parenchyma.

Lower Surface of the Blade

In the description that follows, the reader should picture the epidermis as if the long axis of the leaf or stolon were lying left to right across the field of view of the microscope and the short axis up and down in the field of view. When the epidermis is this viewed the left to right axis will be referred to as horizontal, the axis lying up and down as vertical (Metcalf, 1960).

The lower epidermis of the leaf blade consists principally of long cells in costal and intercostal zones except in those rows in which stomata

occur. The rows are more or less straight, although some undulation results from distortions around widened stomatal complexes. Walls of long cells are thin and pitted. The long horizontal walls are sinuous. Short end walls are usually straight.

Short cells occur within many of the rows of long cells. These are more frequent in the rows of long cells lying over the principal veins. Four or five of the rows of long cells overlying a major vein may contain short cells, while only a single row of cells over lesser veins may include short cells.

Over the veins most of the short cells occur singly and alternately with long cells. Most of them contain silica bodies which are saddle-shaped and occupy the entire lumen of the cell. Hence, the shape of the cell and of its contents are the same. The silica bodies frequently contain one or more dark spots. Commonly short cells occur in pairs in which case only the distal member of the pair contains a silica body (Fig. 17).

Stomatal cells alternate with long cells in rows along each side of the costal zones. One long cell lies between each complex of stomatal cells. A single row of cells containing stomata borders each side of each vein, and the rows run more or less continuously throughout the length of the blade. The stomata are thus confined to the sides of the ribs overlying the veins.

The ovoid complex of cells which surrounds the stomatal aperture consists of guard cells plus two subsidiary cells (Fig. 17). Guard cells have the dumbbell shape characteristic of the Gramineae with enlarged lumina on each end and a constriction in the middle. The aperture of the stoma is a narrow, oblong slit between the constricted mid-sections of the

opposing guard cells. The larger subsidiary cells adjacent to the guard cells are each dome-shaped thus giving the characteristic oval form to the stomatal complex. The walls of subsidiary cells are smooth and thin, lacking the sinuous appearance of those of adjacent long cells.

Long cells between the stomatal complexes are wider than those in neighboring rows. The end walls of these long cells are concave adjacent to the stomatal complexes (Fig. 17). Horizontal walls of the interstomatal long cells are thin and sinuous as in other long cells.

Macrohairs are long, slender, straight and unicellular with thick walls and acuminate tips (Fig. 12, 22). The base is sunken in a mound of irregular-shaped, enlarged epidermal cells (Fig. 22). In transection these basal cells resemble the large, thin-walled, colorless cells that make up the bulliform tissue of the leaf. Macrohairs occur in the intercostal zones where they are interspersed among the microhairs (Fig. 12,22). They occur also on the margins of the blade, and they are especially numerous around the collar.

Microhairs are bicellular consisting of one tapering basal cell four to six times longer than the short dome-shaped distal cell (Fig. 17, 18). The distal cell is thick-walled and colorless and does not stain. The wall of the basal cell is not uniform; the side adjacent to the distal cell is several times thicker than the other sides.

Microhairs are found in both the costal and intercostal zones where they appear irregularly in the strips of cells between those bearing stomata. The constricted basal portion of the basal cell is inserted between two short cells, two long cells, or between one short and one long cell.

Pricklehairs are unicellular, pointed trichomes along the margins of the blade (Fig. 12, 19). They are thick-walled, colorless and are commonly preceded by a short cell and followed by a long cell in the marginal row. Numerous deviations from this pattern occur. Two pricklehairs may be found adjacent to each other or only a single long cell may lie between two of them. Pricklehairs extend beyond the margin of the blade at about a 30 degree angle, the extended portion usually representing one-third of the total length of the cell.

Upper Surface of the Blade

The principal type of cell in the upper epidermis of the blade is the thin-walled long cell (Fig. 12, 18). The walls are sinuous on the long axis and mostly straight on the short axis. Long cells lie in parallel bands over the veins with bulliform cells in rows between the veins. Saddle-shaped cells occur between the long cells. Over the minor veins, only a single row of long cells includes saddle-shaped short cells, each of which contains a silica body filling the entire lumen.

On each side of a vein is a band of cells in which stomatal complexes alternate with the long cells. Both the stomatal complexes and the long cells are like those on the abaxial surface. Bands containing stomata are adjacent to bands of bulliform cells. Papillae are present on the paradermal surfaces of long cells in the stomatal bands (Fig. 18).

Paired, short, crescentic cells are present on the adaxial surface, as well as microhairs. Both of these are found within the bands of long cells. Microhairs are similar to those on the abaxial surface.

Macrohairs are present in the intercostal zones of the upper

epidermis and attached to mounds of large irregular cells located within the bulliform tissue or partially within the adjacent zone (Fig. 12, 22). They do not occur directly over the veins.

Bulliform cells occur between each vein of the blade (Fig. 12, 18, 26). Those derived from the epidermis are colorless and thin-walled, long, narrow, and parallel to the long axis of the blade (Fig. 18). Their walls are wavy but not sinuous as are those of other long epidermal cells. Those derived from internal cells are larger, irregular, and straight walled (Fig. 23, 24, 26).

In both upper and lower surface views, transverse anastomosing veins are visible (Fig. 11). They run diagonally across the intercostal zones and connect one vascular strand with another.

The Leaf Blade in Transverse View

In a transection of the leaf blade, midway between apex and base, the inconspicuous midrib (Fig. 13) contains a single vascular bundle. This midrib and the major veins each include a mestome, a parenchymatous bundle sheath, and radiate chlorenchyma (Fig. 23). On the abaxial side of the midrib and the major veins the parenchymatous sheath and the radiate chlorenchyma are disrupted by a fiber bridge extending from the epidermis to the mestome (Fig. 23). Secondary veins lack this fiber bridge (Fig. 13, 24). In outline the midrib has the form of an isosceles triangle with rounded vertexes and the apex at the adaxial surface of the leaf (Fig. 13). The two halves of the blade on either side of the midrib are usually symmetrical although the number of veins may not be equal. The blade is uniformly thick except for the abrupt taper at the margins which is limited

to the outermost vein (Fig. 13). The ribs and grooves created by the veins are inconspicuous, though they are slightly more prominent on the abaxial than on the adaxial surface.

Radiate chlorenchyma and parenchymatous bundle sheaths are characteristic tissues of chloridoid grasses as described by Brown (1958). The vascular bundles are surrounded by an inner bundle sheath or mestome, an outer parenchymatous bundle sheath, and by a layer of radiate chlorenchyma. The iodine test on transverse sections of leaves reveals the presence of starch both in the parenchymatous sheath and in the radiate chlorenchyma. The test is positive both in the daytime (5:00 P.M. CST, May, 1967) and after dark (9:00 P.M. CST, May, 1967). Brown reports for other members of this group that the chlorenchyma is only faintly green and contains no starch.

In major veins the mestome consists of a single layer of sclerified cells which closely resemble the cells in the fiber bridge. It is continuous with the bridge on the abaxial side. In some cases the mestome interrupts the outer sheath at the adaxial surface and comes into direct contact with the adaxial fiber bridge. In either case the individual cells which make up the mestome vary in size according to their position in the bundle. Those in contact with the fiber bundle on the abaxial side are of the same size as the cells in that bundle. Those toward the adaxial side of the bundle are larger.

The secondary bundles appear angular in transection (Fig. 13, 24). The large thin-walled parenchymatous sheath cells surrounding the mestome outline their perimeter . The principal bundles, including the midrib — vascular bundle, are round or oval. Their boundaries are also marked by

the parenchymatous sheath.

The xylem in the principal bundles usually includes one or two metaxylem vessels on either side of a single protoxylem vessel. Often the protoxylem vessel is absent even in the principal bundles or, if present, is indistinguishable from the tracheids.

In secondary vascular bundles the mestome consists of cells of variable size (Fig. 24). Small cells on the adaxial and abaxial sides of this mestome resemble those in the mestome of the principal bundles. Larger cells toward the middle are more nearly similar to those of the parenchymatous bundle sheath. Xylem in the secondary bundles is much reduced; phloem is more extensive.

In addition to the fibrous sheath extensions associated with the veins, small fiber strands occur at the leaf margins. These consist of not more than three or four small cells as seen in transection.

Abaxial Surface of the Sheath

The abaxial epidermis consists predominately of long cells in rows extending the length of the sheath (Fig. 14, 20). The horizontal walls are sinuous and the end walls usually straight and vertical.

Short cells are mostly in pairs both over the veins and in the intercostal zones, and are either saddle-shaped or crescentic. Between the veins the distal member of a pair contains a silica body.

Stomatal complexes occur in rows of cells on either side of the veins. Guard cells and subsidiary cells are like those in the blade epidermis as are the long cells between them.

Microhairs occur in the intercostal zones as in the blade epidermis.

They tend to be shorter and with the terminal cell larger in relation to the basal cell.

Macrohairs are also present on this surface of the sheath. They are similar to those on the blade, both in form and arrangement. In addition, appendages resembling macrohairs are present on the abaxial epidermis (Fig 14, 20). These are as short as microhairs, non-septate and colorless, with walls similar to those of macrohairs. They are attached between long cells within the intercostal bands. They are not associated with a multicellular mound of epidermal cells as are the macrohairs, although they have cell walls and pointed tips like those of macrohairs.

Both microhairs and macrohairs are more abundant on the upper abaxial epidermis than near the base of the sheath. In other respects no significant differences appear between the epidermal tissues from the two regions.

Adaxial Epidermis of the Sheath

This epidermis consists largely of strips of long cells (Fig. 15, 21). The individual long cells are larger and the strips straighter than their counterparts on the blade and on the abaxial surface of the sheath. The horizontal and end walls are smooth. The end walls are vertical or oblique (Fig. 21). No apparent differences exist between cells in strips over and between veins. Short cells are absent except toward the margins of the sheath, where they occur singly and in pairs. These marginal short cells are narrow and often crescentic. There are few stomata. Neither macrohairs nor microhairs are present on the surface, and there are no prickles on the margins.

There are no apparent differences between the epidermis at the lower and upper ends of the sheath.

The Leaf Sheath in Transverse View

The margins of the leaf sheath overlap from the base to the collar except that the upper margins are forced apart by the growth of enclosed leaves (Fig. 16). A transection of the shoot midway between the base and the collar reveals a series of concentric leaf sheaths encircling a series of developing leaf blades. Two or three leaf sheaths are present in such a transection.

The leaf sheath lacks ribs and grooves such as occur in the blade. There is no distinguishable midrib, although undulations may be present on the adaxial surface of the outermost sheath. They are irregular on the abaxial surface and do not correlate with the fascicular and interfascicular regions. The sheath is thickest at the midsection and tapers very gradually toward the margins.

The mesophyll of the leaf sheath consists of relatively large, thin-walled and colorless parenchyma cells. Conspicuous intercellular spaces are present. The largest portion of the leaf sheath consists of mesophyll tissue. While most of the cells within this region are circular in outline, those toward the adaxial epidermis are angular as if they were compressed. Intercellular spaces also are smaller toward the adaxial surface of the leaf and are entirely absent from that part of the mesophyll immediately adjacent to the adaxial epidermis.

The amount of mesophyll tissue in the sheath gradually diminishes toward the margin. The penultimate margin consists only of the adaxial and

abaxial epidermal layers. The margin itself is often reduced to a single layer of epidermal cells (Fig. 9).

Each vascular bundle in the leaf sheath is surrounded by a bundle sheath of thick-walled, sclerified cells (Fig. 25). This consists of one or more layers of smaller cells on the abaxial side of the bundle and a single layer of larger thick-walled, sclerified cells on the adaxial side (Fig. 25). On the abaxial side of the bundle a sheath extension approaches the epidermis, and on bundles toward the thin margins of the leaf sheath it is in contact with the epidermis.

The vascular bundles are collateral as is typical of grasses. The xylem consists usually of a single pair of lateral metaxylem cells and one or two protoxylem vessels lying toward the middle of the strand. In some instances a protoxylem vessel member is obliterated, and only a cavity surrounded by tracheids remains. A remnant of the secondary thickening occasionally persists within the cavity.

A band of fibers or fiber tracheids separates the xylem from the phloem in most of the bundles. The smaller bundles may consist almost entirely of a sheath of fibers several layers thick surrounding a few phloem elements and tracheids.

A thin layer of sclerenchyma lies between the epidermis and the mesophyll along all or part of the abaxial side of the leaf sheath. This sclerenchyma forms a bridge with the fiber sheaths surrounding the lateral vascular bundles.

Morphology of the Stolon

Stolons are 1 mm in diameter or slightly less, round and smooth.

They are dark green except near the basal end of each internode where they are pale green or light yellow.

Under the dissecting microscope narrow dark green bands paralleling the length of the stolon can be seen. In transections each band appears as a small green crescent lying along the perimeter of the stem. Collectively the bands form a single discontinuous layer. The stolon is usually solid except that the older internodes near the basal end are "pithy" toward the center or occasionally hollow.

Anatomy of the Stolon in Transverse View

Differences occur in the anatomy of the stolon at various levels between nodes.

The arrangement of tissue in a transection from immediately behind the node reflects the latters proximity to the point of emergence of the leaf sheath above (Fig. 27). Beneath the sclerified epidermis is a layer of parenchyma within which are embedded the vascular strands, both median and lateral, from the node above. The median strand lies over a parenchymatous gap in the sclerenchyma cylinder, a band several cells thick beneath the parenchyma layer. The two principal lateral strands are partially embedded within the cylinder. Three pairs of smaller strands lie opposite each other within the parenchymatous layer but outside of the cylinder. The median and principal lateral strands are collateral, and each has a fiber bundle on the outer side.

Immediately below the node, the sclerenchyma cylinder is complete except for one parenchymatous gap subtending the median trace to the leaf above (Fig. 27). The individual cells of this layer are heavily

sclerified. In the ground tissue inside the sclerenchyma layer are numerous irregularly shaped, poorly defined vascular bundles.

A transection midway between nodes reveals a different pattern (Fig. 28). The epidermal layer is like that near the node and is underlaid by a narrow fiber layer beneath which are crescents of radiate chlorenchyma followed by the single layered, thin-walled, parenchymatous sheath cells. The parenchyma and chlorenchyma form a double crescent of cells overlying small but distinct vascular bundles consisting of phloem elements surrounded by fibers. The parenchyma and chlorenchyma pattern is reminiscent of that found in the leaf. The fibers are continuous with the sclerenchyma cylinder and islands of parenchyma occupy regions between the radiate chlorenchyma.

Collateral vascular bundles form an irregular ring inside the sclerenchyma cylinder. A pair of larger bundles lying at opposite poles within the pith are continuations of median bundles in leaves at higher nodes. The bundle nearer the sclerenchyma cylinder is from the leaf at the first node above (Fig. 28). Vascular bundles in the sclerenchyma cylinder and pith are similar to those in the leaf sheath; they lack a bundle sheath. The solid pith consists of large, round parenchyma cells with prominent intercellular spaces.

Above a mature node the stolon lacks the crescents of radiate chlorenchyma and parenchymatous sheath cells (Fig. 31). Beneath the epidermis there is a layer of parenchyma three to four cells thick. Inside this layer is a sclerenchyma cylinder surrounding the thin-walled parenchymatous pith. Scattered throughout the pith are vascular bundles similar to those

found in the leaf blade. Several bundles are connected with the sclerenchyma cylinder by a fiber bridge. Other bundles are completely surrounded by parenchyma.

A transection of an immature stolon above the node has a smaller amount of sclerified tissue (Fig. 30). The sclerenchyma cylinder is narrow and the parenchyma extensive. Scattered collateral bundles contain rows of protoxylem elements or protoxylem lacunae.

Stolon Epidermis

The stolon epidermis consists principally of long cells similar to those found in the leaf (Fig. 29). They have sinuous horizontal walls and lie in strips paralleling the long axis. Rectangular or slightly crescentic short cells occur in pairs alternating with the long cells. They lack silica bodies and are seldom saddle-shaped like the short cells in the leaf epidermis. There is no consistent morphological difference between the distal and the proximal member of the short cell pair as commonly occurs in the leaf. No costal and intercostal zones are present. Stomata occur in rows overlying the chlorenchyma. The guard cells and subsidiary cells form complexes that are similar to those in the leaf blade. Dermal appendages are rare or entirely absent and there is no bulliform tissue.

These characteristics apply throughout the stolon although near its base stomata are less frequent than toward the distal end.

FIGURES 1 - 6

1 Transection through shoot meristem just below apex showing youngest leaf encircling the meristem apex. Four progressively older leaves encircling the youngest one. X112

2 Transection 88 μ below fig. 1 at second internode. X112

3 Transection 114 μ below fig. 1 at third internode. X112

4 Transection 192 μ below fig. 1 at fourth internode. X112

5 Transection 600 μ below fig. 1 at fifth internode. X112

6 Longisection through shoot meristem. X275

Key to abbreviations:

Cm central meristem

L1 leaf number one

L2 leaf number two

L3 leaf number three

lpb2 major lateral procambial bundle of leaf number two

lpb3 major lateral procambial bundle of leaf number three

lpb4 major lateral procambial bundle of leaf number four

mb3 minor bundle of leaf number three

mb4 minor bundle of leaf number four

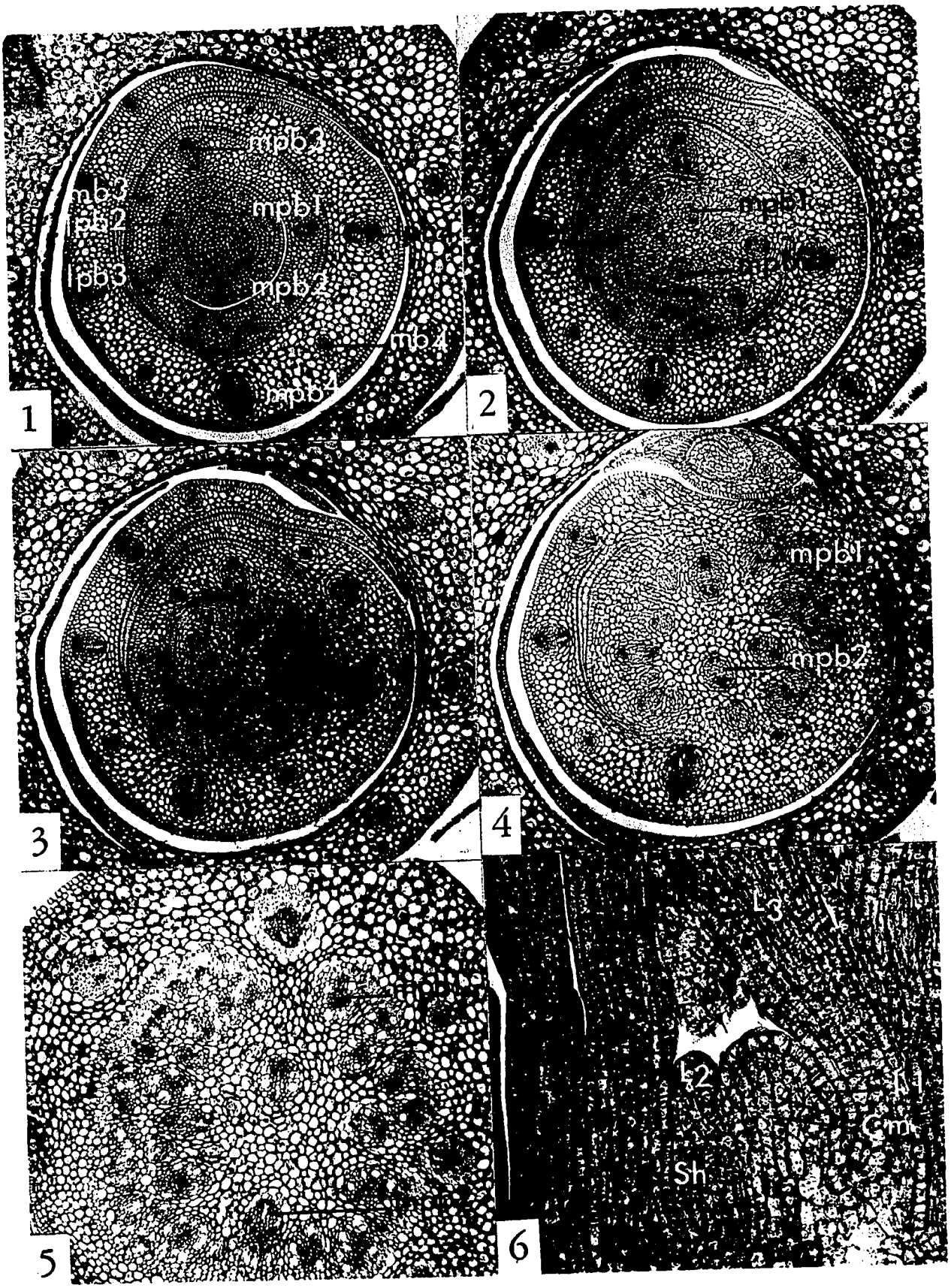
mpb1 median procambial bundle of leaf number one

mpb2 median procambial bundle of leaf number two

mpb3 median procambial bundle of leaf number three

mpb4 median procambial bundle of leaf number four

Sh sheath of leaf number three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FIGURES 7 - 10

7 Transection through shoot meristem just below apex showing two youngest leaves encircling meristem apex. X575

8 Transection through median procambial bundle of third from the youngest leaf and adjacent leaf segments. X575

9 Transection through lateral procambial bundles of third (right) and fourth (left) from the youngest leaves. X575

10 Transection through median procambial bundle of fourth from the youngest leaf. X575

Key to abbreviations:

bs bundle sheath

inv invaginations at bulliform regions of second from the youngest leaf

ep double epidermis of margin of developing sheath of fourth from the youngest leaf

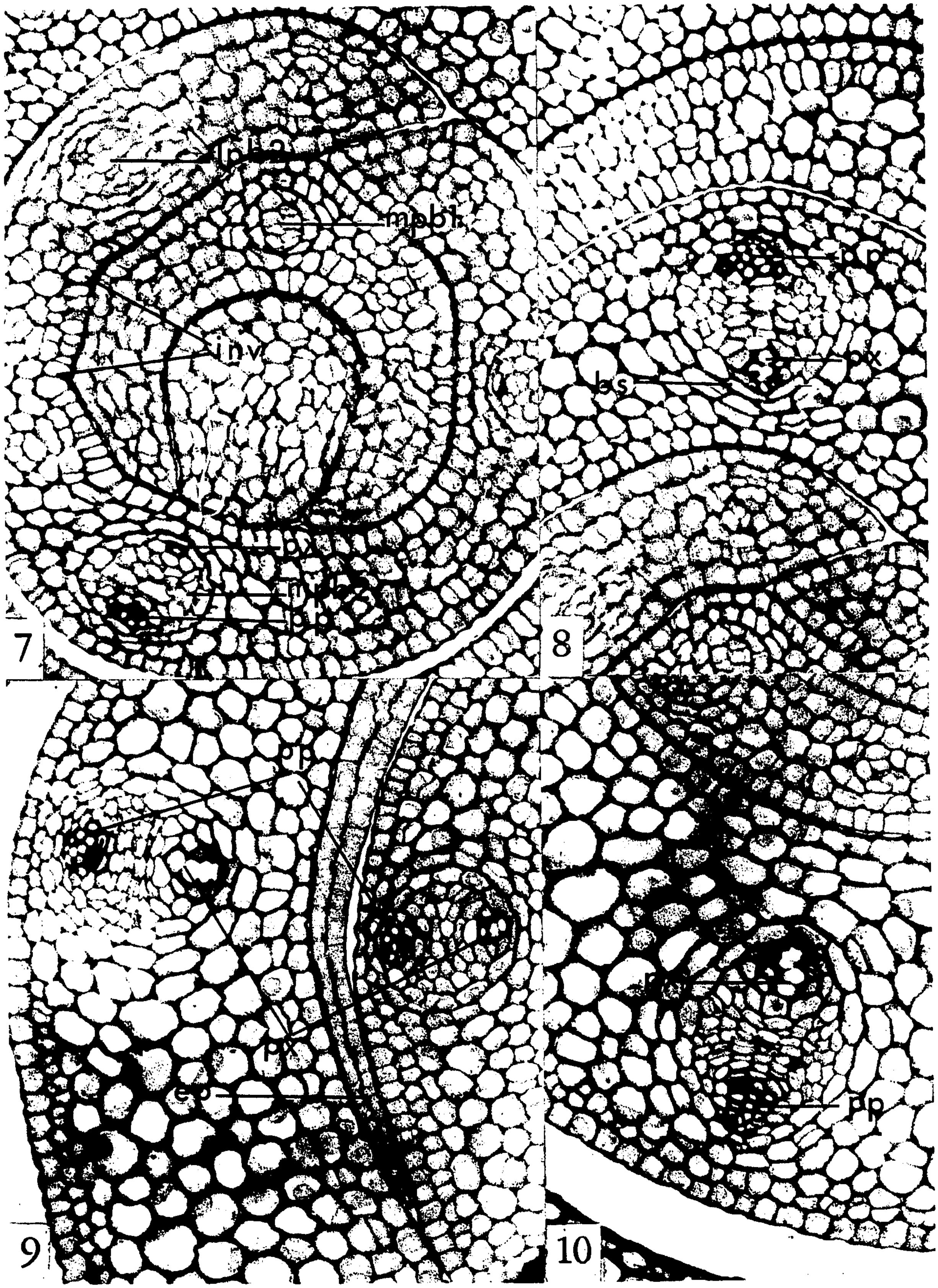
lpb2 same as in fig. 1

mpb1 same as in fig. 1

mpb2 same as in fig. 1

pp protophloem

px protoxylem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FIGURES 11 - 16

11 Abaxial blade epidermis. X112

12 Adaxial blade epidermis. X112

13 Transection of leaf blade. Adaxial side toward the left. X140

14 Abaxial sheath epidermis. X112

15 Adaxial sheath epidermis consisting of large thin-walled long cells. X112

16 Transection of two leaf sheaths enclosing two younger blades. Note undulations on abaxial surface of outer sheath. X82

Key to abbreviations:

av anastomosing veins

bl bulliform cells

cz costal zone

iz intercostal zone

mb major bundle

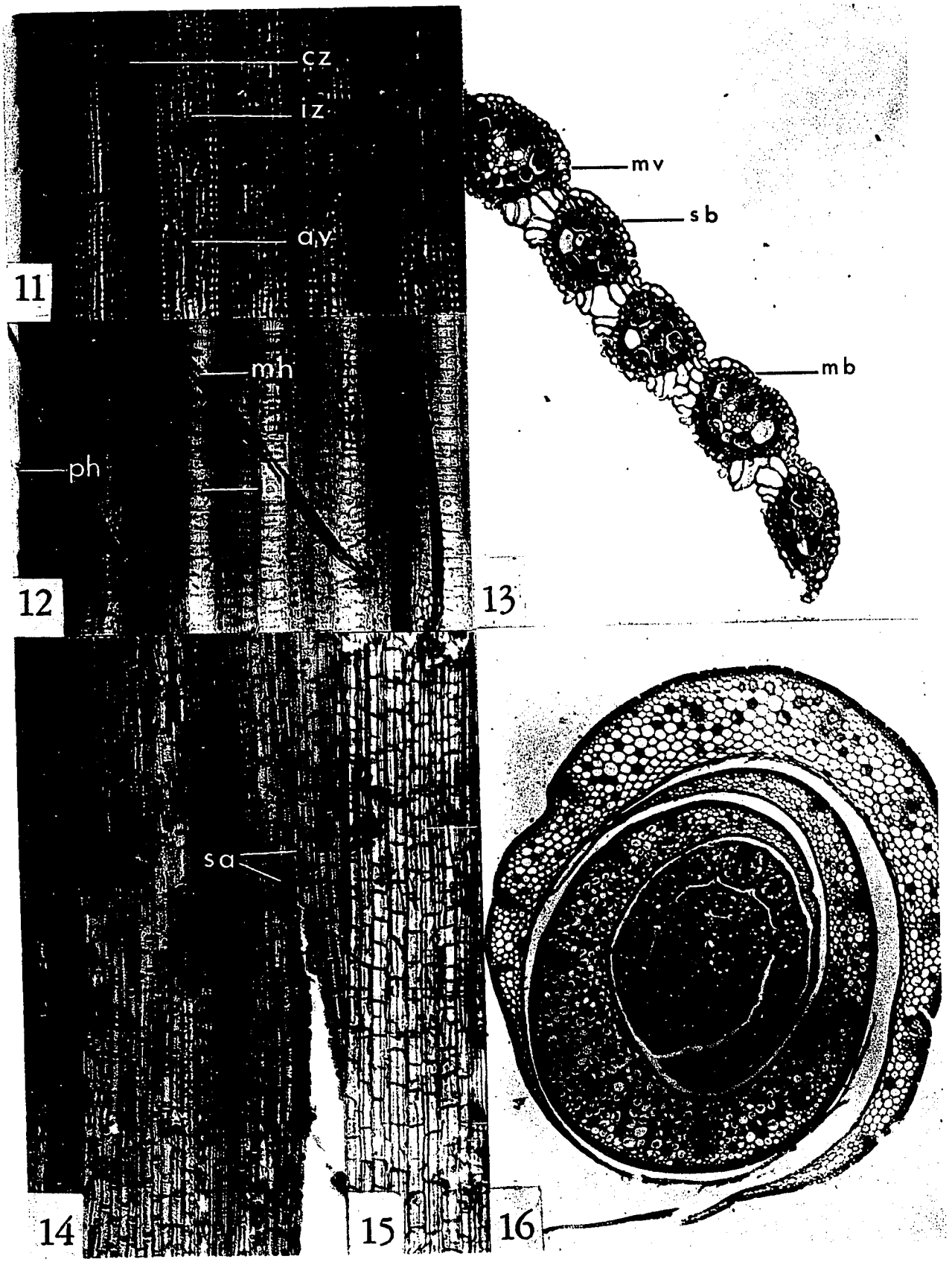
mh macrohair

mv midvein

ph pricklehair

sb secondary bundle

sa short appendages resembling macrohairs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FIGURES 17 - 22

17 Paradermal view of abaxial blade epidermis. X575

18 Paradermal view of adaxial blade epidermis. X575

19 Pricklehairs. X575

20 Paradermal view of abaxial sheath epidermis. X575

21 Paradermal view of adaxial sheath epidermis. X575

22 Paradermal view of abaxial blade epidermis. X230

Key to abbreviations:

bl bulliform cells

bm basal mound of cells of macrohair

ce concave end wall

cs crescentic short cell

lc long cell

mih microhair

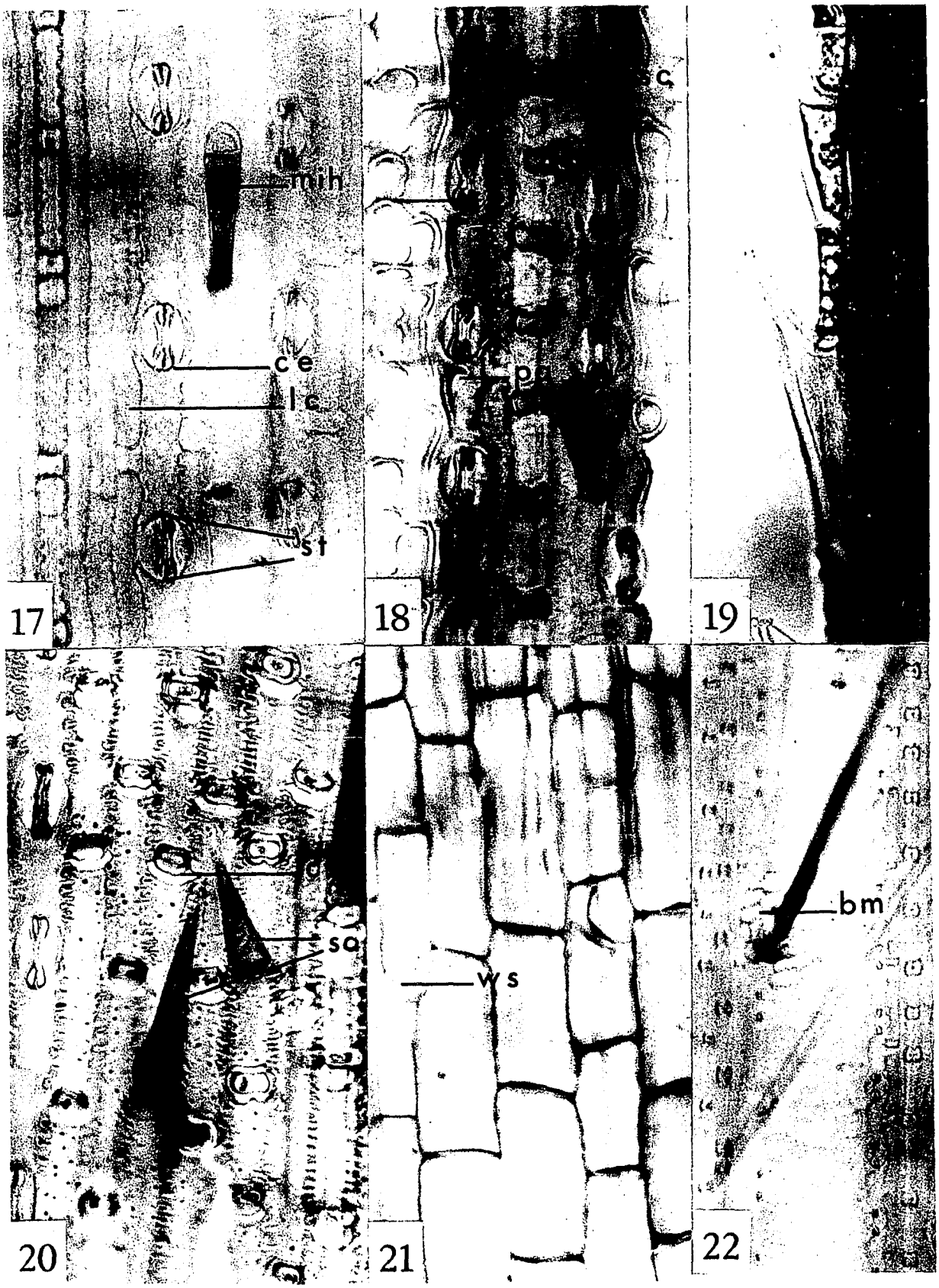
pa papillae

sa short appendages resembling macrohairs

ssc saddle-shaped short cell containing silica body

st stomatal complex of cells

ws wedge-shaped end walls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FIGURES 23 - 26

23 Transection of major vascular bundle of leaf blade. Note larger cells in mestome toward adaxial side; small ones toward abaxial side. X575

24 Transection of a secondary vascular bundle of leaf blade. X575

25 Transection of major vascular bundle of leaf sheath. X575

26 Transection of developing vascular bundle of very young leaf blade. X575

Key to abbreviations:

bl bulliform cell

bs bundle sheath

fb fiber bridge on abaxial surface

is inner sheath of secondary bundle

me mestome

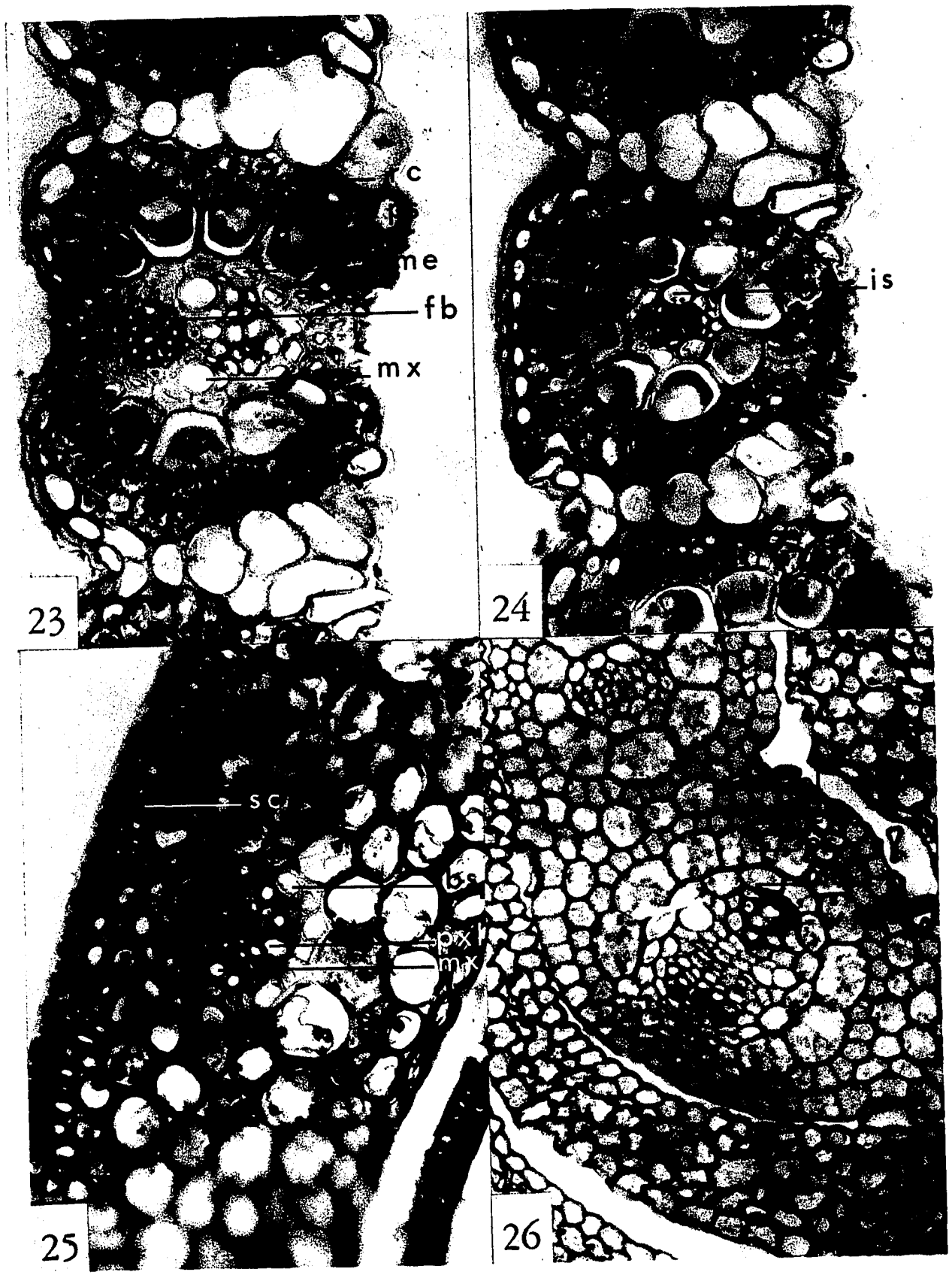
mx metaxylem

ps parenchymatous bundle sheath

pxl protoxylem lacuna

rc radiate chlorenchyma

sc sclerenchyma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FIGURES 27 - 31

27 Transection of stolon immediately behind node. Note parenchymatous gap in sclerenchyma cylinder at median vascular bundle from node above. X112

28 Transection of mature stolon midway between nodes. X140

29 Stolon epidermis showing long cells with sinuous margins, paired and single short cells and stomatal complexes. Darker vertical areas lie over bands of radiate chlorenchyma. X112

30 Transection of stolon immediately above an immature node. X112

31 Transection of stolon immediately above a mature node. The sclerenchyma cylinder is more extensive than in figure 30. X112

Key to abbreviations:

fb fiber bundle of median vascular bundle from node above

lb major lateral bundle

mb median vascular bundle from node above

mib minor bundles from node above

mx metaxylem

pa parenchyma

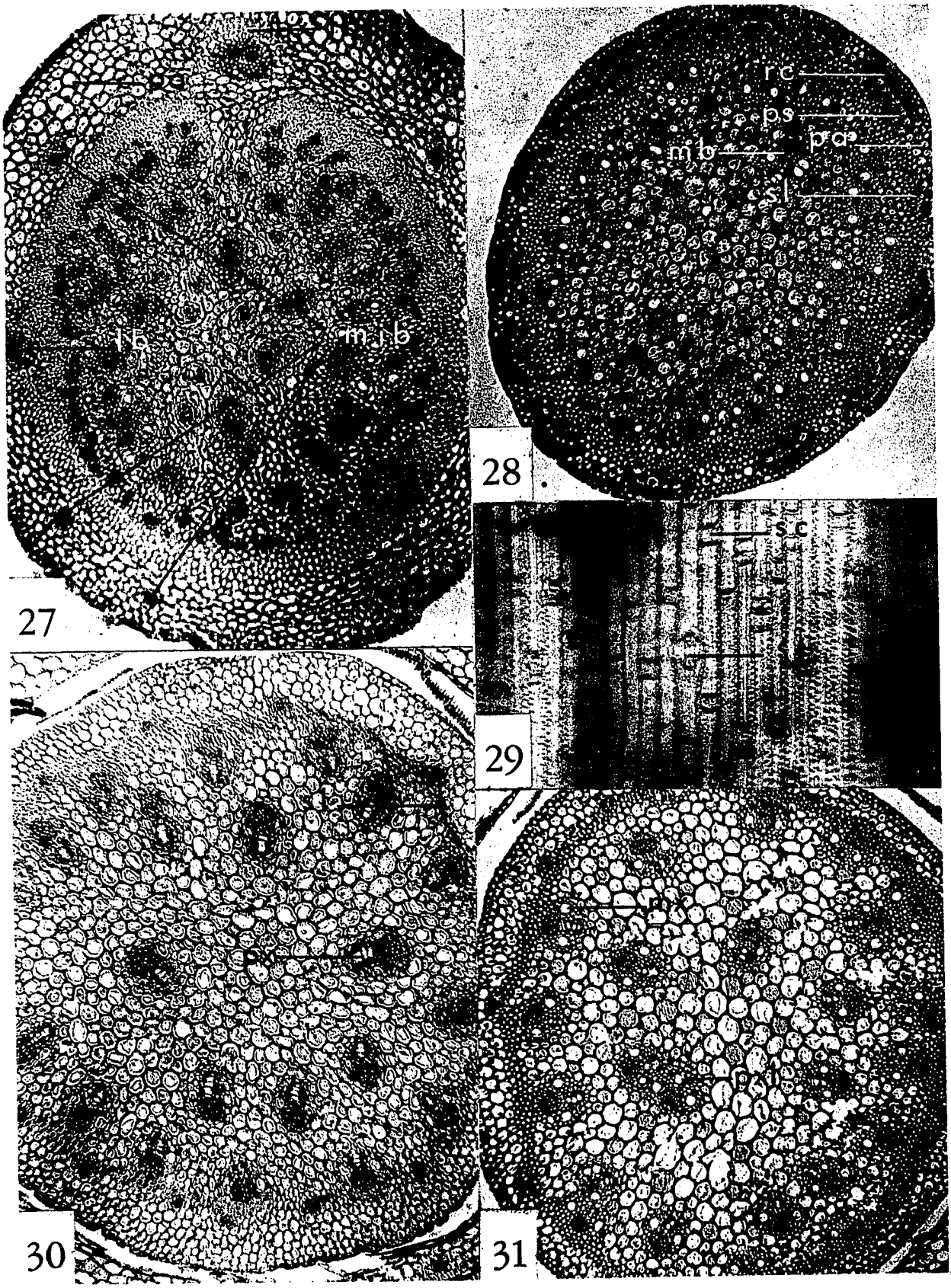
ps parenchymatous sheath cells

px protoxylem

pxl protoxylem lacuna

rc radiate chlorenchyma

sl sclerenchyma cylinder



27 28 29 30 31

CHAPTER IV

SUMMARY AND DISCUSSION

Investigation of leaf and stolon anatomy of buffalo grass reveals a number of significant characteristics. The epidermal cells are quite diversified. Long cells both over and between veins constitute the basic cell type on all surfaces investigated. The long cells all possess sinuous horizontal walls and usually vertical end walls except on the adaxial side of the sheath. In the upper epidermis of the blade the continuous parallel rows of long cells are replaced in the intercostal zones by strips of bulliform cells. Long cells in the adaxial epidermis of the sheath have straight, thin walls. The strips of long cells on this surface are not interrupted by short cells or dermal appendages except toward the margins.

More diversity of form and distribution exists in short cells than in long. Short cells over veins of the blade are usually single and saddle-shaped. Between veins on the lower leaf surface short cells in rectangular or crescentic pairs are common. This paired arrangement is also common in the outer sheath epidermis and on the epidermis of the stolon. Short cells are present only toward the margins of the inner sheath epidermis.

The various types of dermal appendages - macrohairs, microhairs, and pricklehairs - are similar in shape and distribution on all surfaces

examined. The single exception is the occurrence of very short hairs resembling macrohairs on the outer sheath epidermis. These are attached between pairs of long cells and are not associated with mounds of epidermal cells as are typical macrohairs.

Papillae were noted only on long cells which occur between stomatal complexes and adjacent to bulliform tissue.

The vascular tissue in the leaves and the stolon bears significant similarities and differences. Vascular bundles of the leaf blade have radiate chlorenchyma and parenchymatous bundle sheaths. The bundles, including the secondary and median, possess a mestome. Bundles in the leaf sheaths lack these two distinctive parenchymatous layers although sheath bundles are enclosed in one or more layers of sclerified cells. The vascular system in the mature stolon midway between internodes consists of components similar to the system in both the leaf sheath and the blade. The small vascular bundles lying outside the sclerenchyma cylinder of the stolon are overarched by semicircular patches of radiate chlorenchyma and parenchymatous bundle sheath cells reminiscent of the vascular bundles within the leaf blade. These small vascular bundles arise de novo behind the internode. They traverse the internode outside the fiber cylinder and disappear above the basal node without connection to the vascular bundles lying within.

Vascular bundles from the leaves pass through the node and into the stolon where they appear inside the sclerenchyma cylinder. The midrib from the leaf above becomes one of the two large vascular bundles lying nearer the center of the pith in each internode. The median bundle of the

youngest leaf can be traced to a position inside and adjacent to the sclerenchyma cylinder of the youngest elongated internode. Although in the mature leaf blade these vascular bundles are surrounded by radiate chlorenchyma and parenchymatous bundle sheaths and are continuous with vascular bundles in the sheath and stolon, in the latter organs these distinctive tissues are absent.

The fundamental tissue of the leaf blade consists principally of radiate chlorenchyma, the parenchymatous bundle sheaths and the internal bulliform tissue. The principal fundamental tissue of the leaf sheath is parenchyma which is continuous with the cortical layer at the node. The cortex is extensive only immediately above and below the node. Between nodes it is reduced to small islands of parenchyma between the radiate chlorenchyma. Pith is present between nodes. It is usually solid although it may become "pithy" or even hollow in some older stolons.

Leaf blade anatomy of buffalo grass is chloridoid as defined by Brown (1958). It is exceptional in that the radiate chlorenchyma of the leaf is richly endowed with chloroplasts, rather than scarcely endowed as Brown reports for other members of the tribe. These chloroplasts may be of a different type from those found in the parenchyma sheath as is suggested by their slightly different shade of green.

The recurrence of radiate chlorenchyma in both the stolon and the leaf blade indicates the fundamental similarity of these two organs. A study of the ontogeny of this tissue in both stolon and blade would shed additional light on this similarity. Examination of leaf blades and stolons of other members of the tribe to see if the similarity is consistent throughout the tribe would be of interest.

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