LIGULE IN *SELAGINELLA DELICATULA* (DESV.) ALSTON[1]

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Abstract: The anatomy and ontogeny of ligule in *Selaginella delicatula* (Desv.) Alston were examined using both optical and electron microscopes. The ligule varies in form, from finger-like, bi- to multilobed palmate, acropetally as four regions basing on the cell morphology. They are sheath, glossopodium, basal cell region and neck. The sheath cells as well as the glossopodial cells are transfer cell in the well developed ligule. The former is flattened double cell layered the latter is single row of elongated and highly vacuolated cells. Basal cell region is composed of relatively isodiametric protoplasmic cells whereas the cells in neck are overlapped each other in arrangement. The first indication of the senescence of a ligule is the shrinkage of neck cells. The possible function of each region of the ligule is assumed based on cellular structure.

The ligule arises from two to three rows of adaxial protodermal cells at the junction of leaf primodium and stem. As it grows, the basal cells are the first tissue to be identified, followed by glossopodial cells, sheath cells and neck cells.

INTRODUCTION

Ligule is constantly present in both sterile and fertile leaves of *Selaginella* and *Isoetes*. The appearrance of ligule in *Selaginella* has been widely described by Gibson as early as 1896. He pointed out that the ligule in *Selaginella* varies in outline according to the species, from short rectangular plate, tongue-like to fan-shaped with lobed; with or without papillate margin. He has also observed that considerable variation may occur in the precise form of the ligule even in the same plant. But on the whole, within certain limits, the outline is maintained fairly constant for the species.

Hofmeister (1851) is the carliest botanist who has described the developmental pattern of ligule of *Selaginella*. According to his research, the ligule in both *S. delicatula* and *S. qualeottei* originated from a double row of cells at the base of young leaf, but the ligule in *S. martensii* has been found to arise from a single row of four to six cells (Pfeffer, 1871). Though the different observations concerning with the number of initial cell were reported the sites of the initial cells in the majority of species were described as at the junction of the stem and leaf base (Gibson, 1896). The study on the origin and development of ligule in *Selaginella* has been made very early, but no detailed reports are presented in recent publication.

Ligule has been studied primarily with regard to its structure and development, but not exactly its function. It is thought to be a secretory structure merely based on its cellular structure. As a matter of fact, various peculiar characters

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in the cells of ligule of *Selaginella* related to secretion have been observed (Dunlop, 1949; Gibson, 1896; Horner, Beltz, Jageles and Boudreau, 1975; Kohlenbach and Geier, 1970; McNab, 1887). The same prediction has also been supported by several electron microscopic surveys (Horner *et al.*, 1975; Sigee, 1974, 1975, 1976). The secreted substance associated with ligule has been detected histochemically in several species of *Selaginella* (Bilderback, 1987; Bilderback & Slone, 1987). However, there is still some controversy about the nature of secretion in ligule of *Selaginella.* The purpose of this study is to trace the origin and developmental dattern in *S. delicatula,* and to study ultrastructure for cytoplasmic organelles which have been received much attention by recent workers (Horner, 1975; Bilderback and Slone, 1987).

MATERIALS AND METHODS

All leaves of *Selaginella delicatula* (Desv.) Alston were taken from the plants grown in the shadding house. The plants have been cultivated for more than two years after removing from the field. Proper materials, especially the various leaf ages, were removed from the plants for sectioning. For SEM, the leaves were fixed with 2.5% glutaraldehyde and 1% OsO4, dehydrated with acetone series, then were critical-point dried. Having been dissected, the specimens were mounted on stub, coated with gold and viewed in Hitachi S-520 SEM. For OM and TEM, materials were fixed with the same fixatives as that in SEM. After dehydrated, embedded in Spurr's resin, cut with a glass knife on an ultramicrotome and stained with 0.1% toluidine blue O for OM, and uranyl acetate-lead citrate for TEM (Reynold, 1963; Spurr, 1969).

RESULTS

I. Structure of ligule

A well developed ligule may be up to 330 μm width and 190 μm height, and varies in it outline from slender finger-like, tongue-shape to two to several lobed palmate (Figs. 1-6).

Longitudinal section of a mature ligule shows a differentiation of the cells composing it. There is said to be four regions (Figs. 7, 8, 10). From the base to the apex, they are sheath, glossopodium, basal cell region and neck. Sheath is composed of two rows of cells, closely contacted with the epidermal tissue (Figs. 8, 9). The sheath cells vary very much in shape. The cells located at the middle region appear to be more cubical whereas those seated in the margin near the margin of ligule are elongated. The ligule base is closely surrounded by the sheath, being sunken in the leaf tissue. These two rows of sheathing cells are

Key to labelings

Cy—cytoplasm; D—dictyosome; DL—dorsal leaf; Ec—neck cell region; ER—endoplasmic reticulum; Gm—glossopodial mother cell; Gp—glossopodium; Ic—basal cell region; L—ligule; Li—ligule initial cell; Lp—leaf primordium; Me—mesophyll; Mi—mitochondrion; N—nucleus; Pb—protein body; Pd—plasmodesma; N—nucleus; Pb—protein body; Pd—plasmodesma; Pl—plasma-lemma; Pt—plastid; S—starch; Sh—sheath cell (region); Sm—sheath mother cell; Tr—tracheary element; Va—vacuole; Ve—vein.

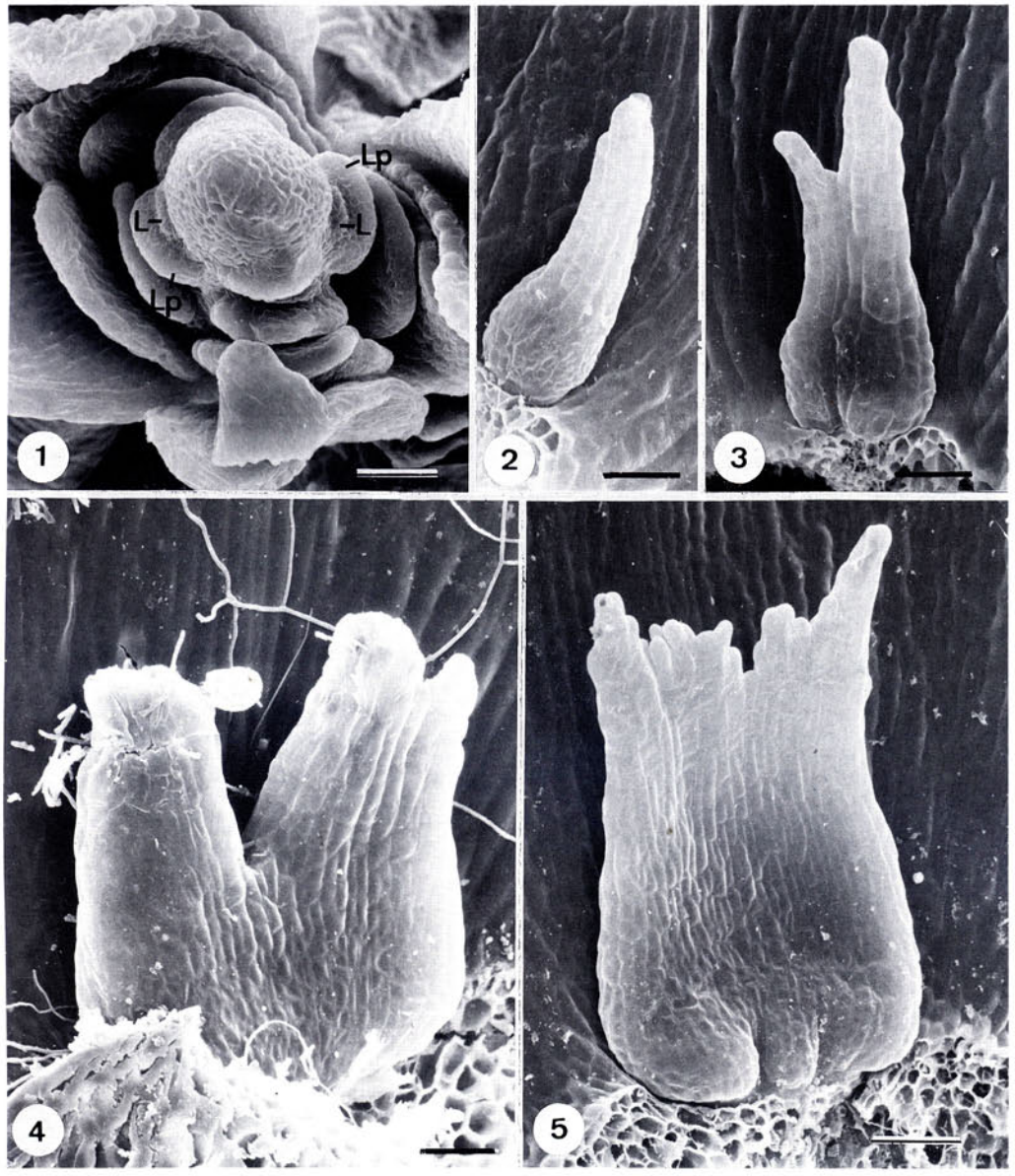
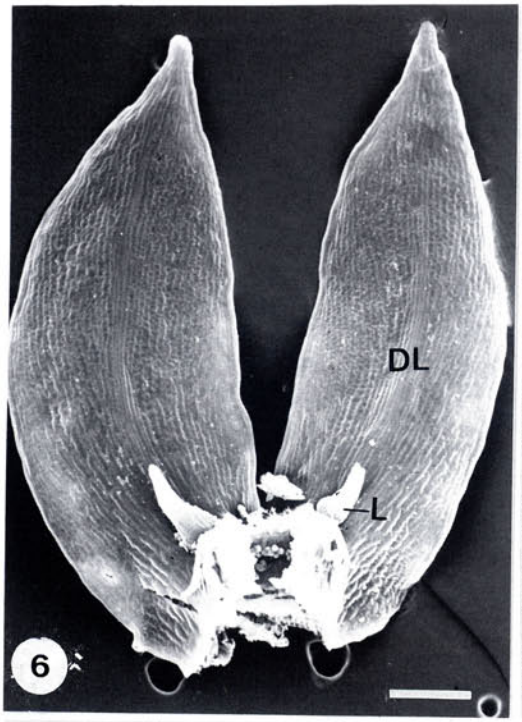


Fig. 1. SEM view of a shoot apex (bar=30 μm).
Figs. 2-5. SEM view various shapes of ligule (bar: 2, 3, 4=35 μm; 5=45 μm).

Fig. 6. The ventral view of dorsal leaves under SEM (bar=150 μm).
Fig. 7. Longitudinal section of a well deveoped ligule and its adjacent tissue (paraffin embedded; bar=60 μm).
Fig. 8. Longitudinal section of a ligule at later stage (plastic embedded; bar=40 μm).
Fig. 9. Enlarged from Fig. 8, showing the basal part of ligule (bar=20 μm).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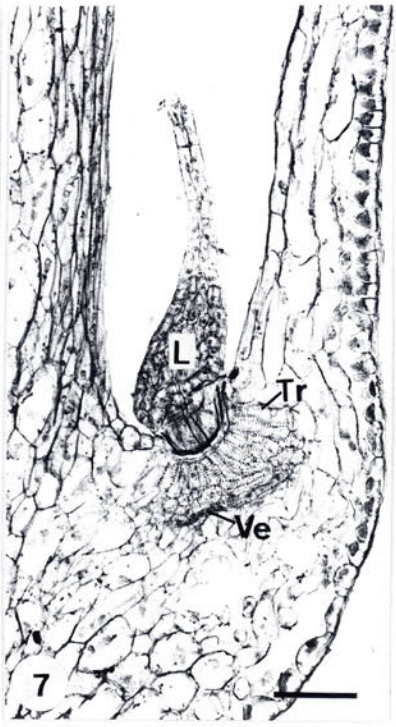
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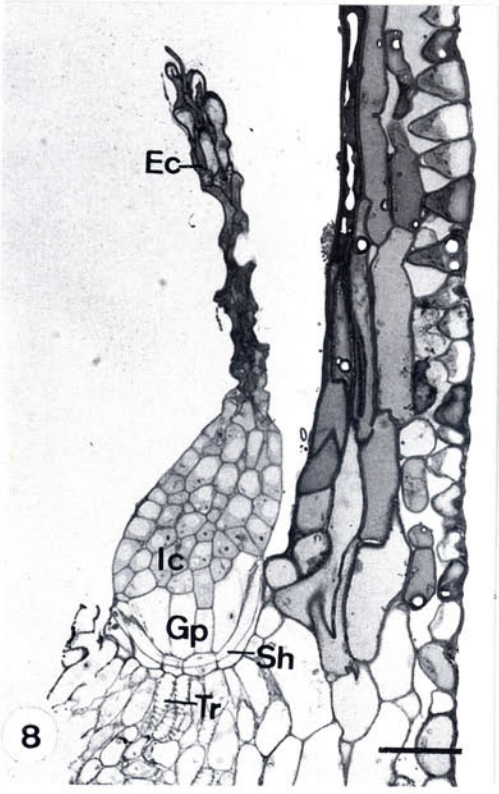
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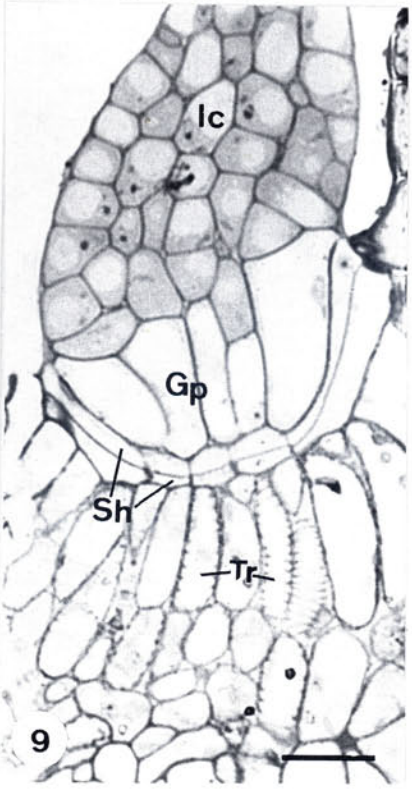
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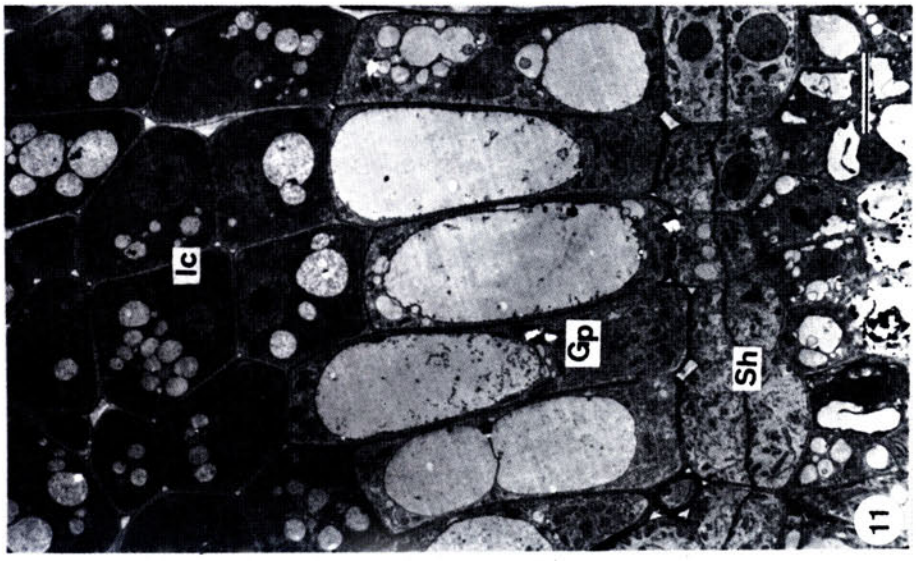


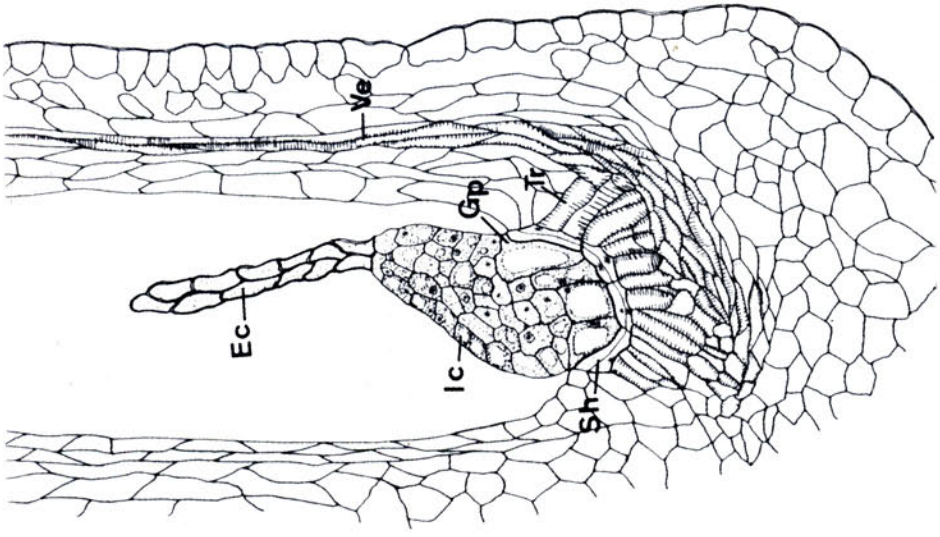
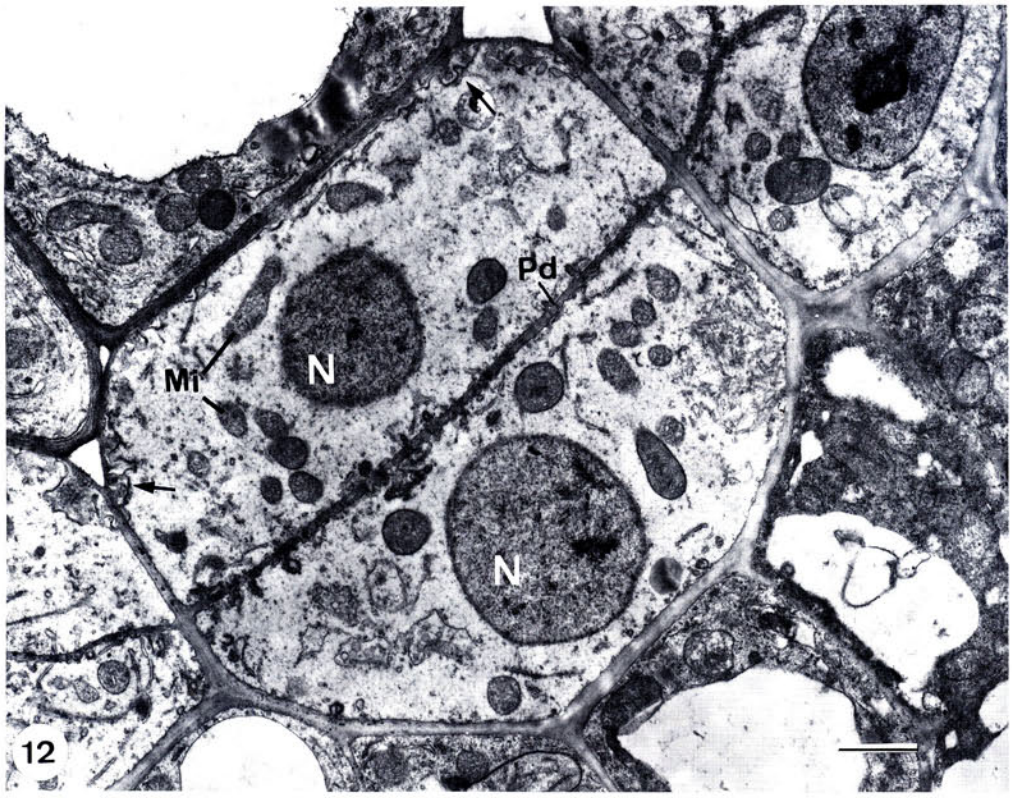


Fig. 10. Schematic drawing showing the relationship of a well developed ligule and its corresponding leaf.
Fig. 11. TEM micrograph showing basal cells, glossopodial cells and sheath cells (bar=1 μm).



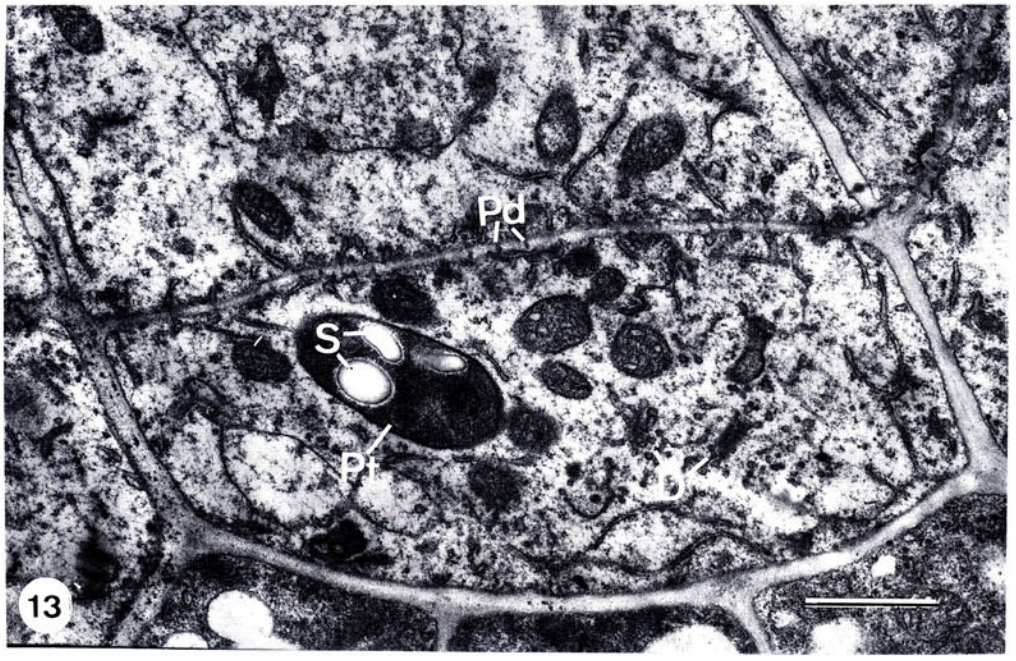


Fig. 12. Sheath cells of a juvenile ligule, note the paired sister cells (bar=1 μm).
Fig. 13. Sheath cells of a juvenile ligule with plastid containing starch (bar=1 μm).

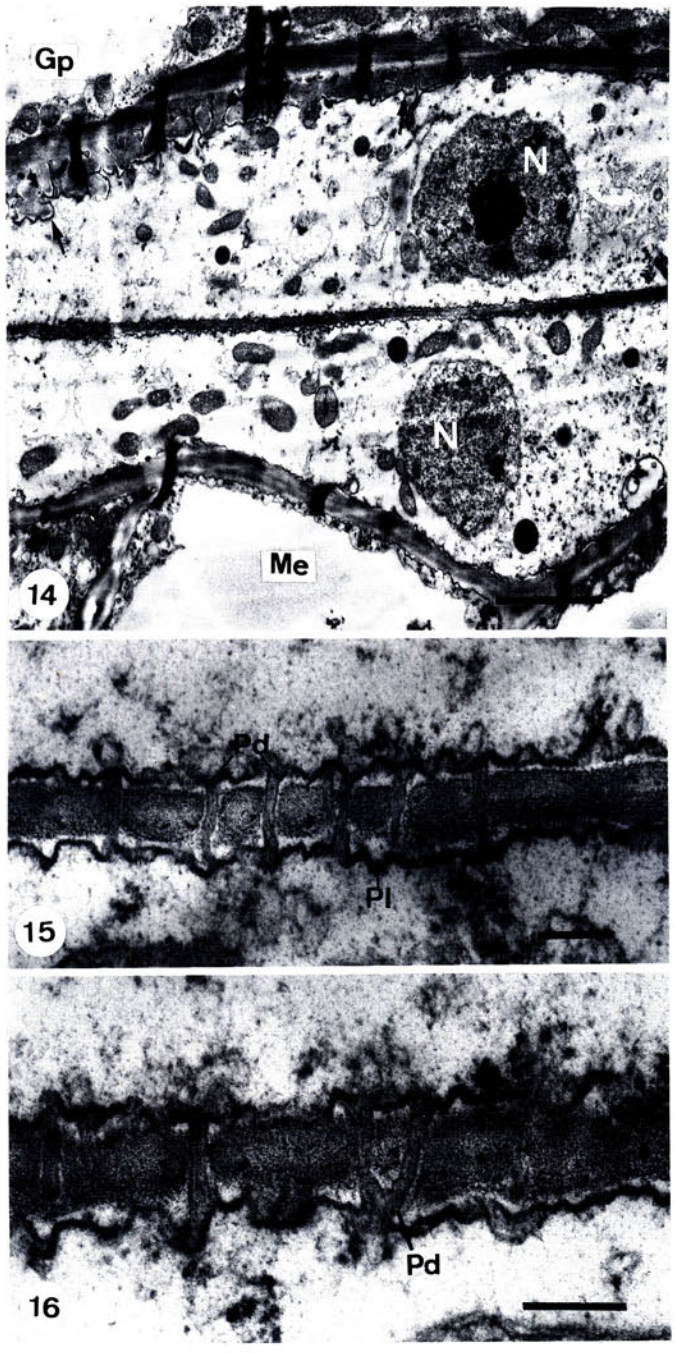
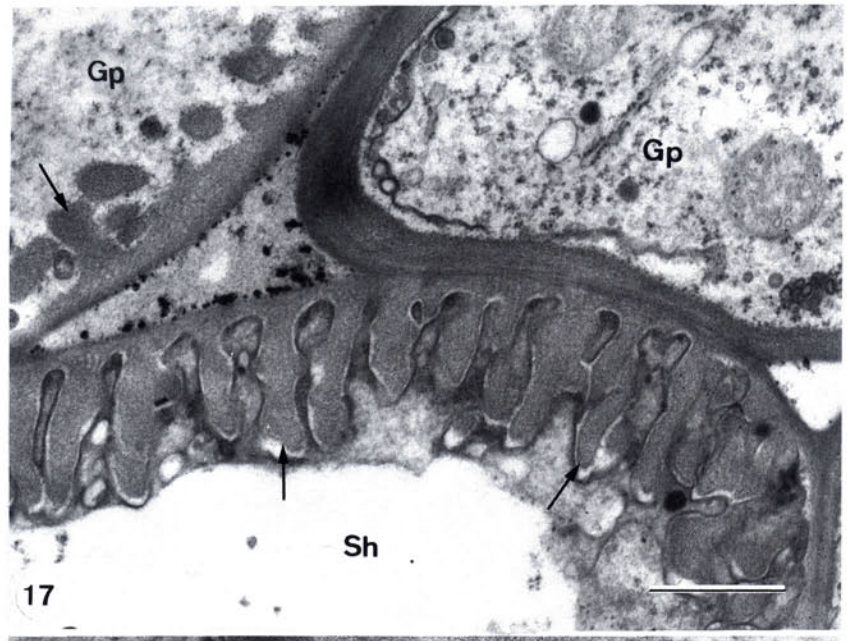


Fig. 14. A pair of sheath cells of ligule at the middle stage, note the initiation of wall ingrowth in lower sheath cell (baa=2 μm).
Fig. 15. Enlarge view of the wall between two sister cells of ligule (bar=0.2 μm).
Fig. 16. Some as in Fig. 15, showing forked plasmodesma (bar=0.2 μm).



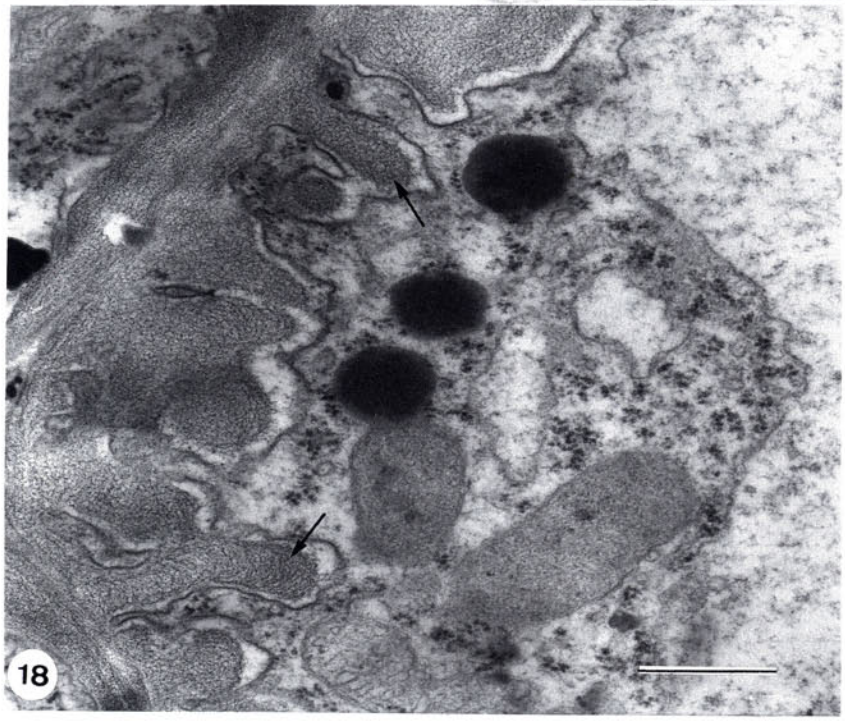
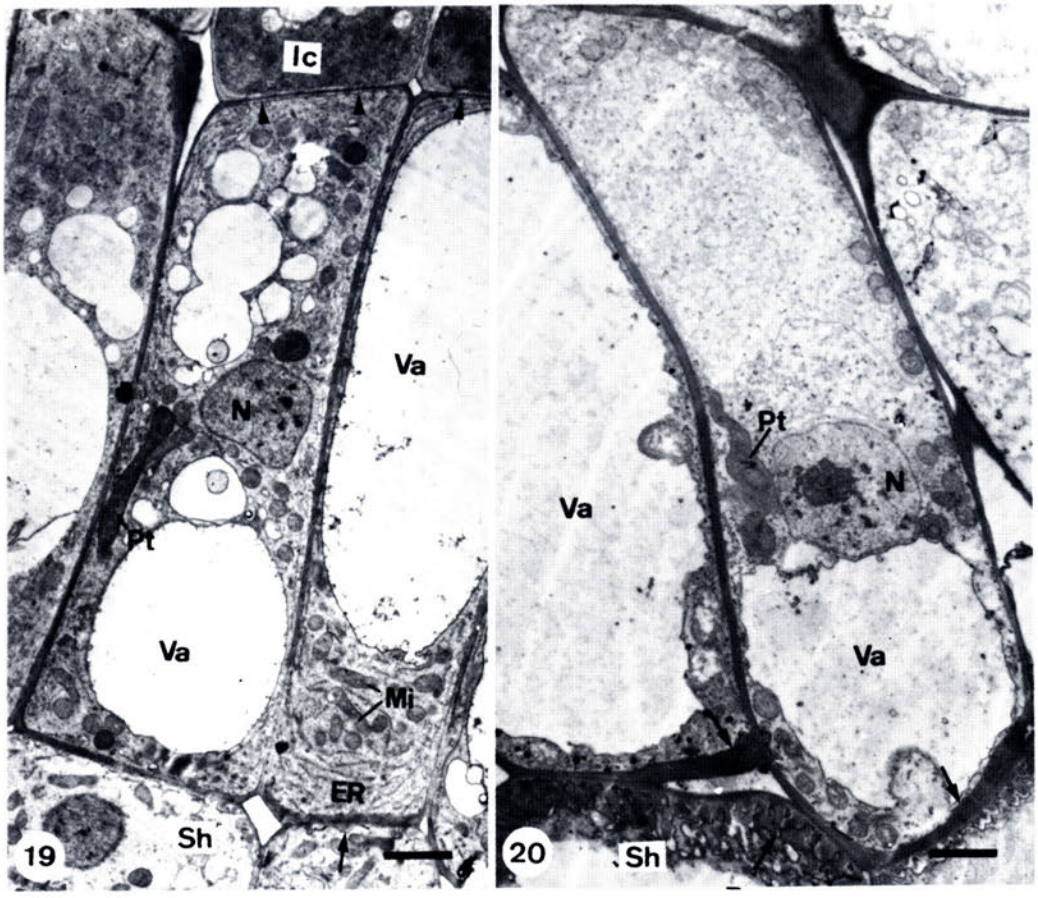


Fig. 17. Thick wall ingrowth (arrow) on outer wall of upper sheth cell and basal wall of glossopodium (bar=1 μm).

Fig. 18. Enlarge view of an outer wall of glossopodial cell showing the wall ingrowth (bar=0.5 μm).

intimately lined each other. In the other words, an individual lower sheath cell and its contiguoue upper sheath cell are paired sister cells. They look alike in outline and the wall in between is always thinner, with more plasmodesmata (Figs. 10-14). Some of these plasmodesmata are branched (Figs. 15, 16). All sheath cells are found to be transfer cells with ingrowth walls, on both inner periclinal wall of lower sheath cell and outer periclinal wall of upper sheath cell (Figs. 11, 17, 18). The wall ingrowth in upper sheath cell is formed prior to that in the lower sheath cell (Fig. 14). The wall ingrowth in upper sheath cell is always thicker than that in the lower sheath cell in a mature ligule. The wall ingrowth is less conspicuous in its young stage (Figs. 12, 13). The nucleus and nucleolus in sheath cell are relatively large. The cells contain deeply stained plastids, often has starch in young stage, numberous mitochondria, sparsely distributed endoplasmic reticulum, and less vacuolated cytoplasm (Figs. 11-13).

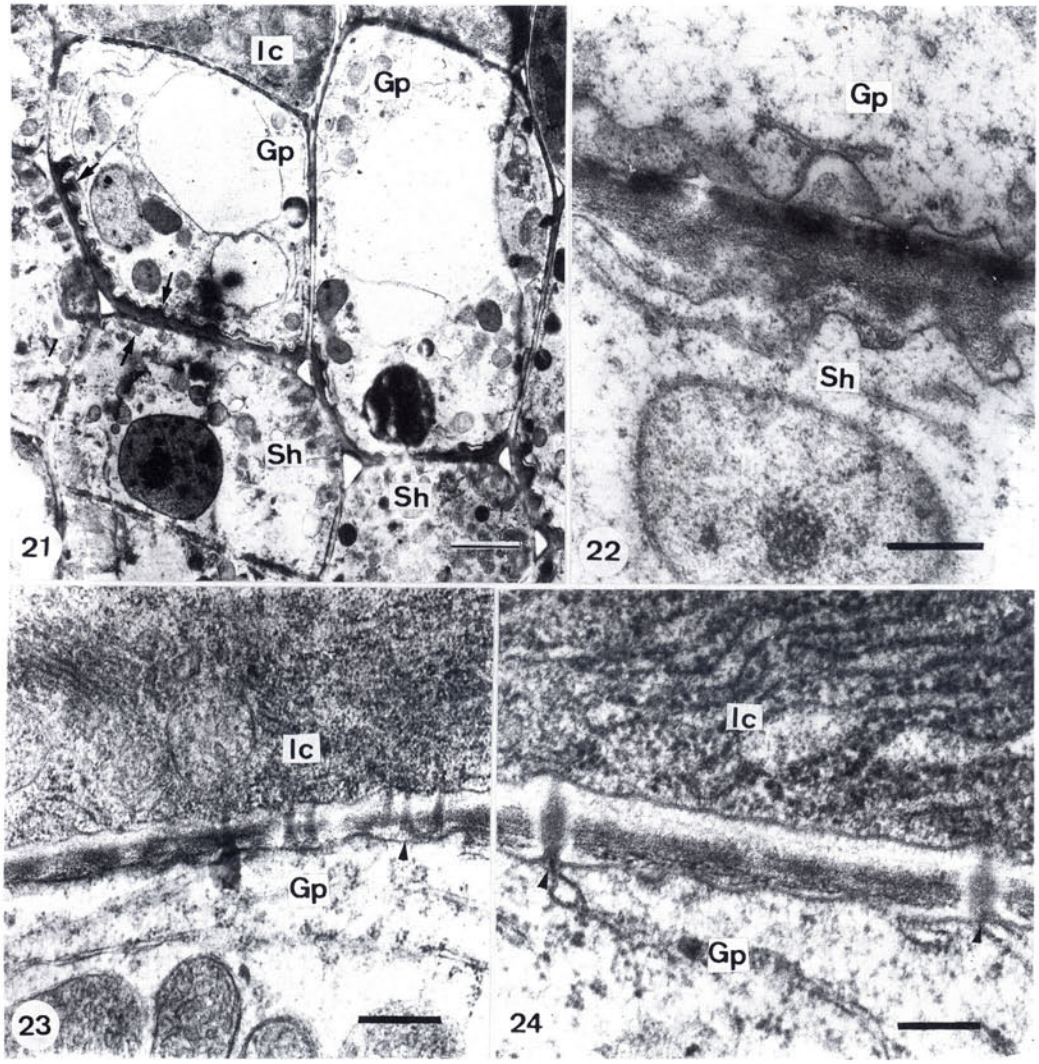
Next to the sheath is glossopodium, a layer of highly vacuolated elongated cells perpendicular to the surface of leaf, enclosed by sheath cells (Figs. 8, 9, 10, 11). Intercellular spaces are formed at the corners of the walls between the sheath cell and glossopodial cell. The glossopodial cell together with the sheath cell are embedded in the leaf tissue. The basal wall (contact with sheath cell)



Figs. 19, 20. TEM of glossopodial cells (arrow: ingrowth wall; arrowhead: plasmodesma; bar=2 μm).

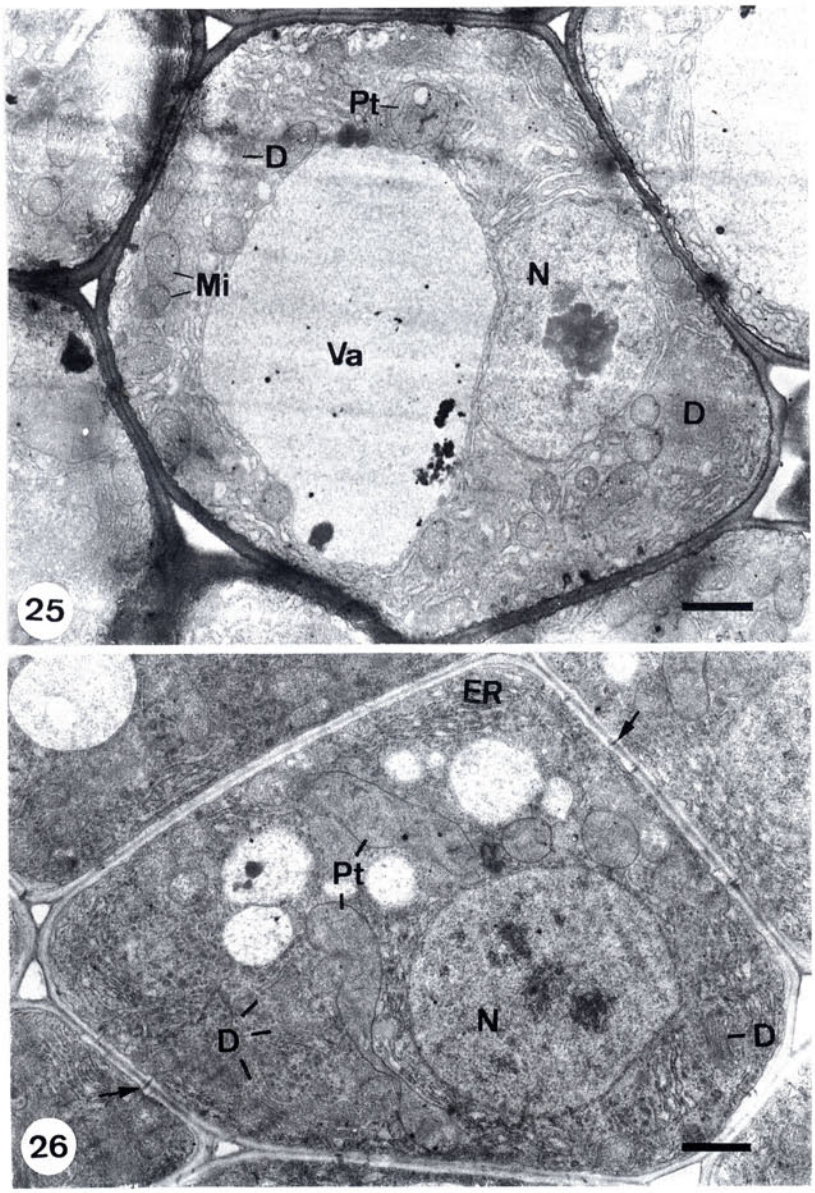
of some glossopodial cells exhibit ingrowth pattern as the transfer cell (Figs. 19–21). Walls are rather uniform in thickness, and no plasmodesmata are found on the wall of two neighboring glossopodial cells. Its upper wall is the only wall bearing plasmodesmata (Figs. 19, 23, 24). The glossopodial cell is the most conspicuously vacuolated and elongated cell among the cells constituting ligule (Figs. 7–10, 19, 20). The majority of the organelles, such as: nucleus, mitochondria and endoplasmic reticulum, retain their figures, but the plastid always shows irregular in its outline (Figs. 19, 20).

Above the glossopodium is basal cell region, forming the thickest and greatest part of the ligule. Its thickest portion consists of five to seven cell layers. The cells are relatively isodiametric, froming intercellular spaces. The cell walls are

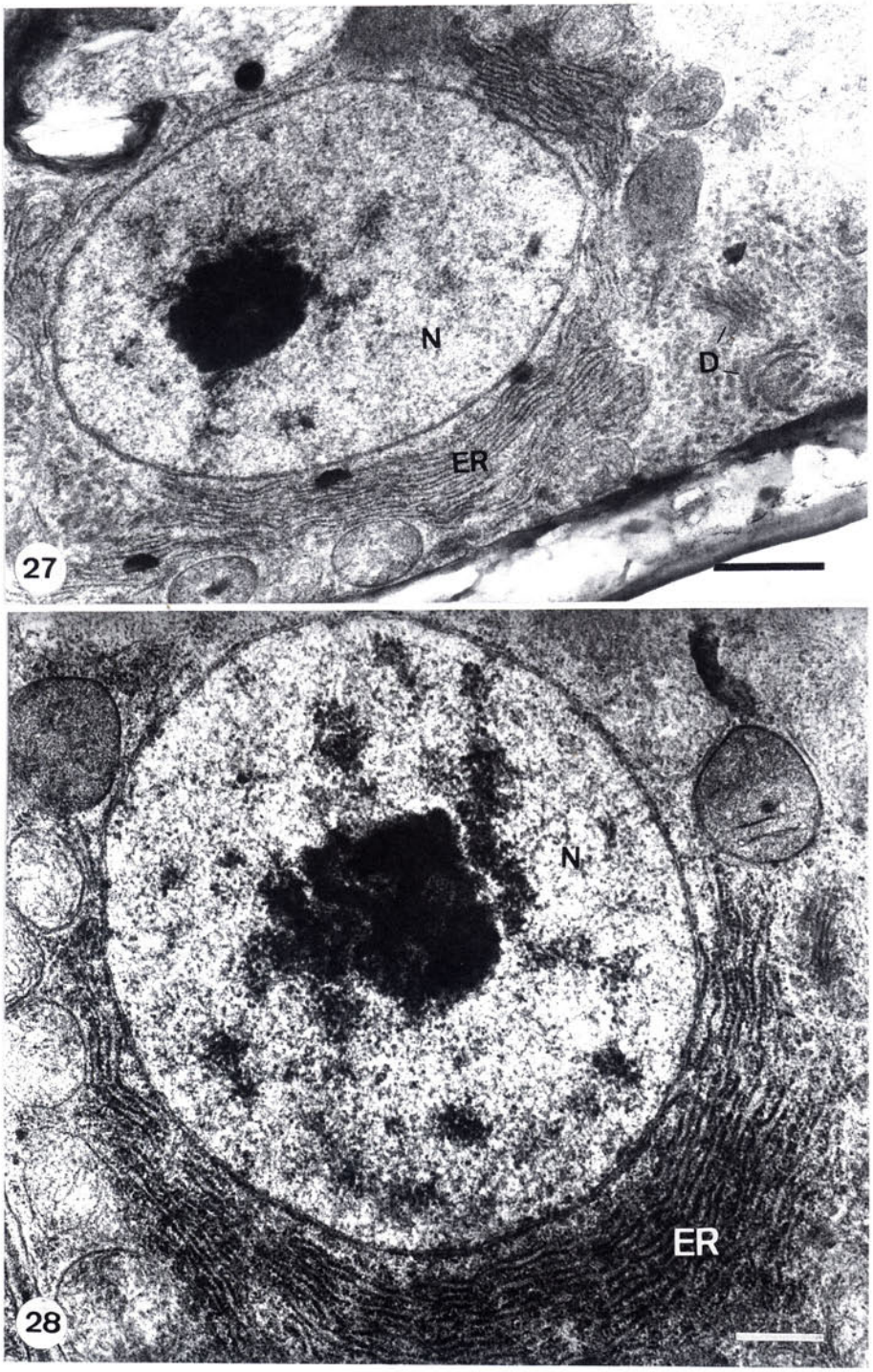


Figs. 21-24. TEM of sheath cells, glossopodial cells and basal cells (arrow: ingrowth wall; arrowhead: plasmodesma; bar: 21=2 μm; 22=0.5 μm; 23 & 24=0.2 μm).

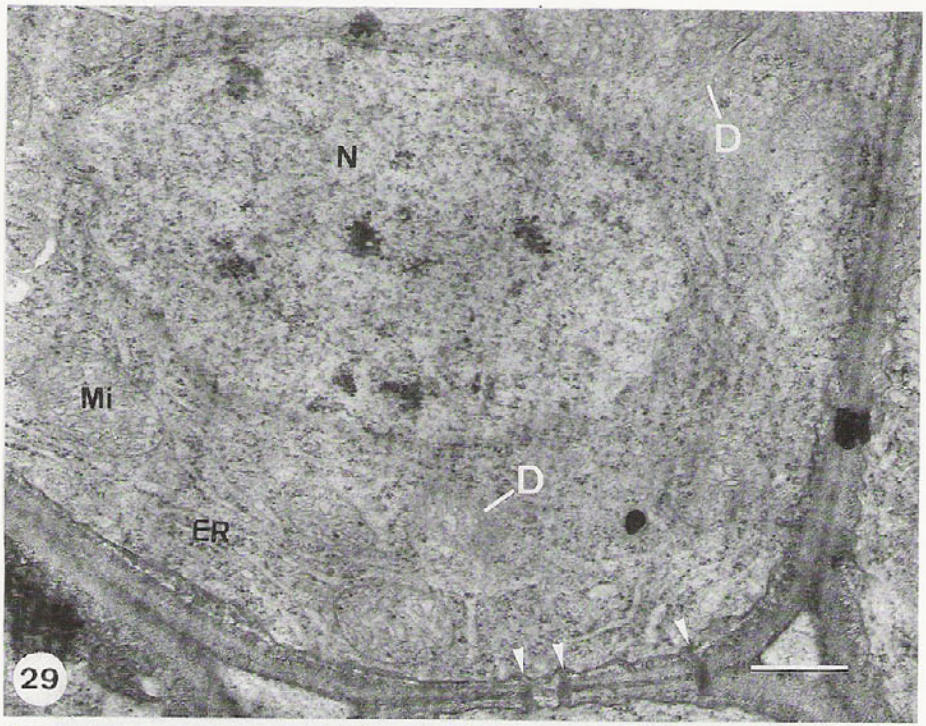
evenly thin bearing numerous plasmodesmata (Figs. 26, 29). The wall that located in contact with the glossopodial cell develops more plasmodesmata than other part (Figs. 23, 24). The protoplasm is dense, deeply stained. In addition to large nucleus, abundant mitochondria, dictyosomes and endoplasmic reticulum, the plastid exhibits irregular in shape as that found in glossopodial cells (Figs. 25-30). But the plastid is characterized by possessing osmiophilic bodies, densely stained plastotubules, loose thylakoids and huge protein bodies (Figs. 30-32). Starch is seen inside the plastid of young cell (Figs. 25, 30, 31).

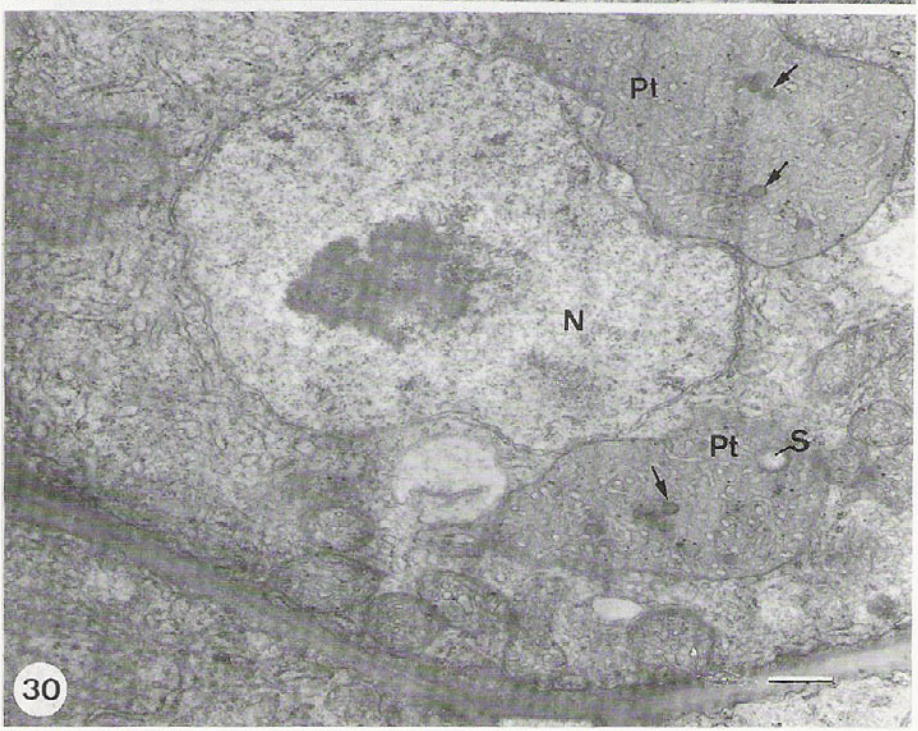


Figs. 25, 26. Basal cells showing various orgenelles (arrow: plasmodesma; bar= 1 μm).

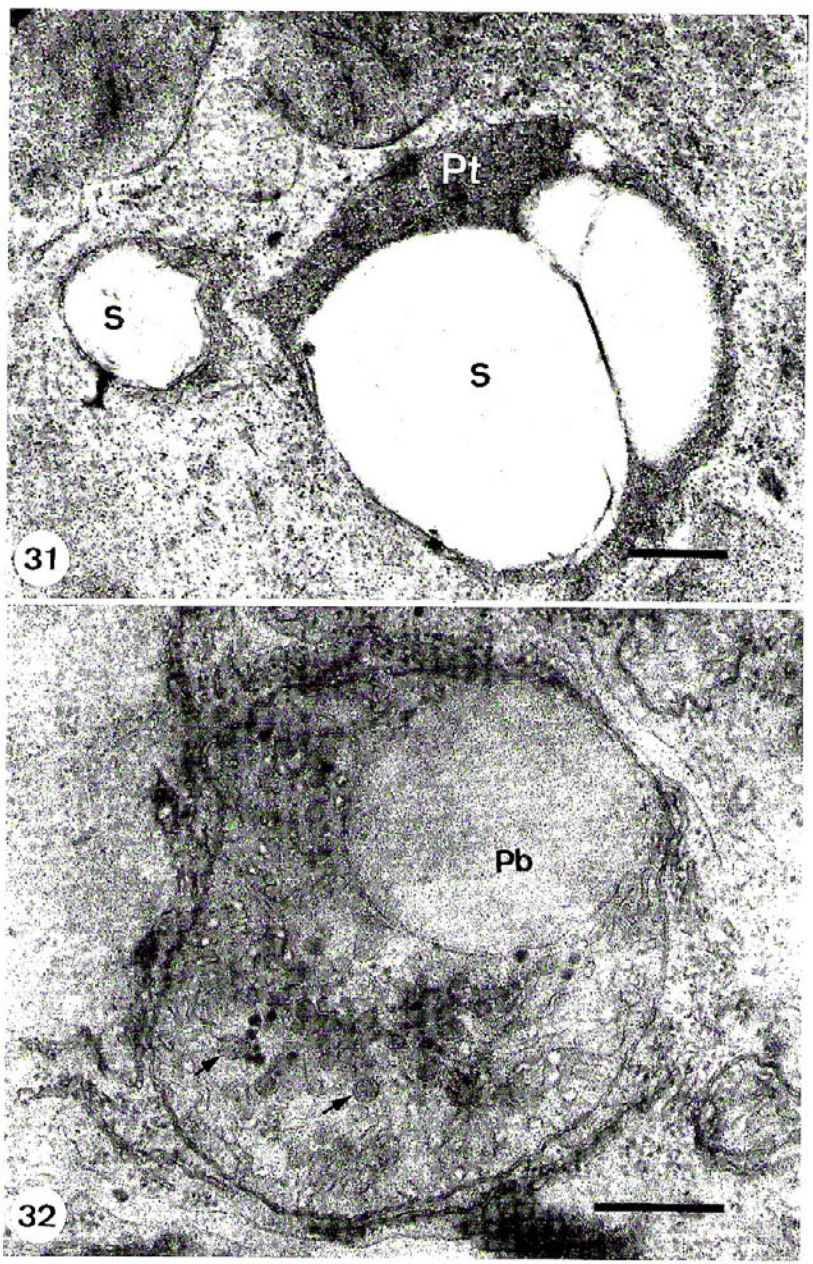


Figs. 27, 28. Basal cells showing the orientation of endoplasmic reticulum (bar= 1 μm).

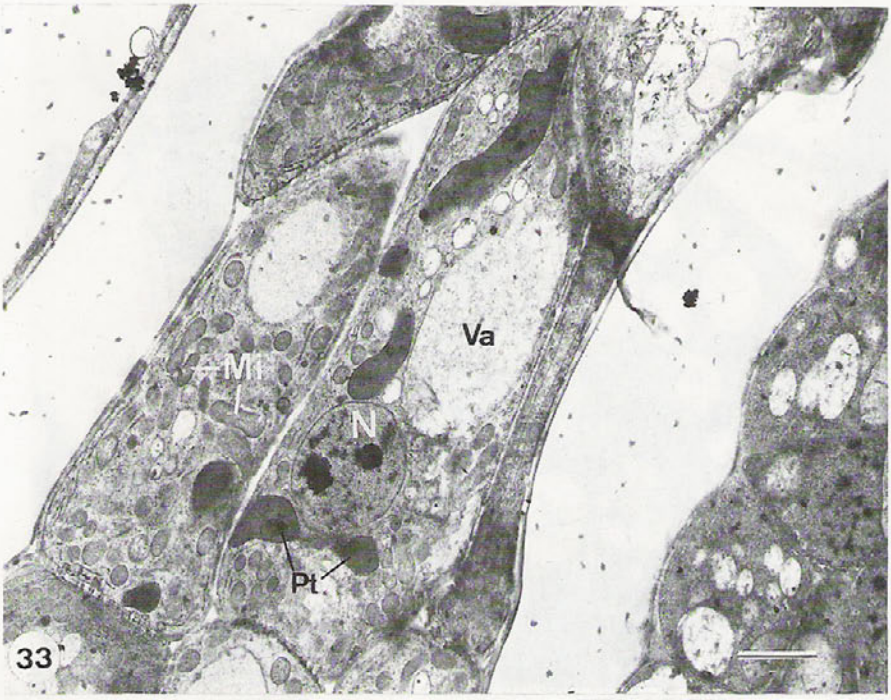




Figs. 29, 30. Basal cells (arrow: plastoglobule; arrowhead: plasmodesma; bar= 0.5 μm).



Figs. 31, 32. Plastids in basal cell (arrow: plastoglobule; bar=0.5 μm).



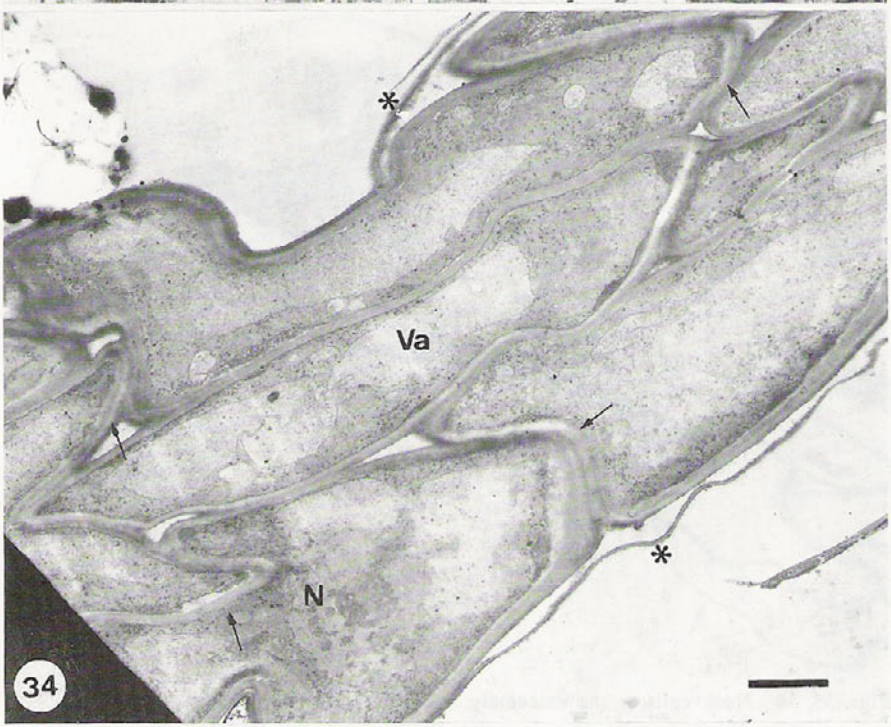
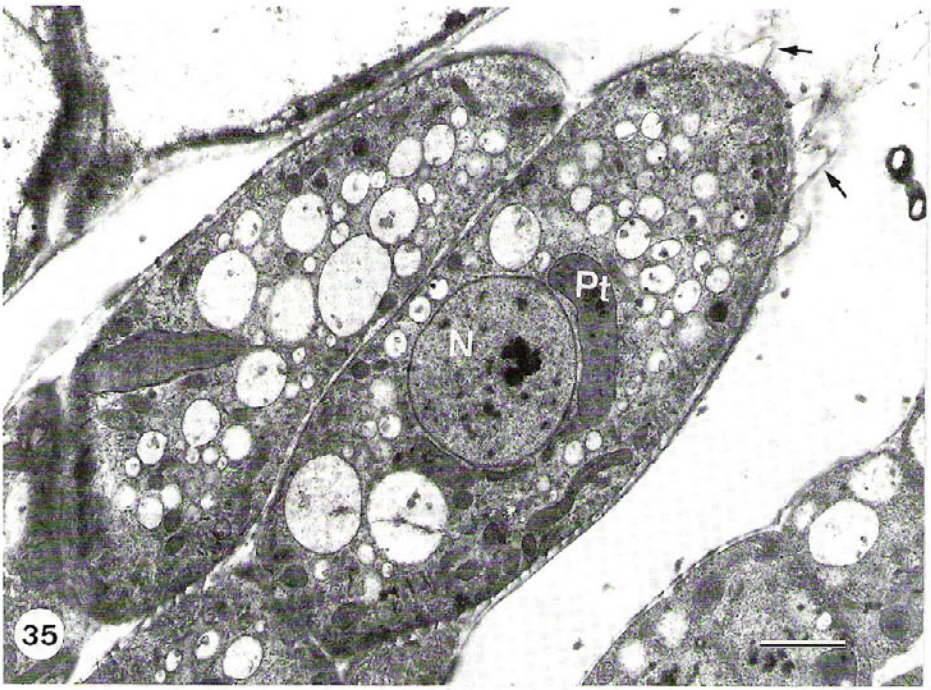
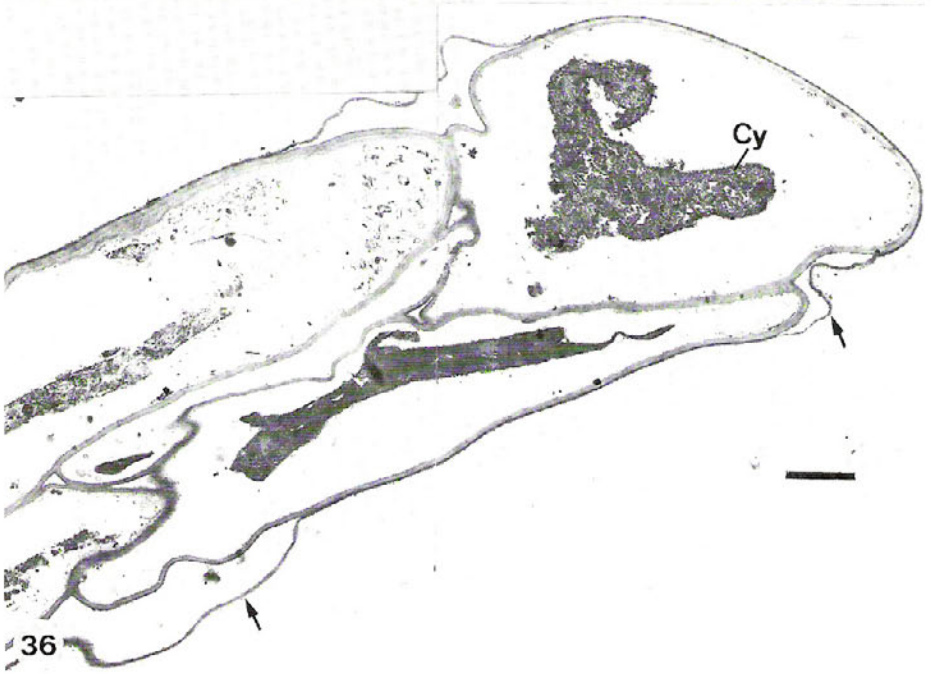


Fig. 33. The neck cells in a juvenile ligule (bar=2 μm).
Fig. 34. The neck cells in a mature ligule, note the sloughing off cuticle layer (*) (bar=2 μm).





Figs. 35, 36. Neck cells at the extremely apex of ligule (bar=2 μm).

Fig. 35. two cells of a juvenile ligule, note the undulate cuticle laye (arrow).

Fig. 36. in an older ligule, note the shrinking cytoplasm and sloughing off cuticle layer.

The apex of ligule constitutes the fourth region, neck, only one to three cell layers in the extreme apex (Figs. 7, 8, 10). The cells are slender in shape and are not arranged in horizontal tier. They are more or less overlapping (Figs. 33, 34).

The ligule is a temporary structure. It becomes withered in later stage. It is pale green in color and more or less transparent, but turns brown at later stage. The shrinkage of neck cells is the first indication of the ligule withering (Figs. 7, 8). The cells in the young ligule consist of relatively dense protoplasm with numerous small vacuoles, mitochondria and plastids (Figs. 33, 34). As a ligule grows, the vacuoles exhibit larger, membrane system of cytoplasmic organelles become obscure and cell inclusions become shrinked (Figs. 35, 36). Finally the breakage of whole cell occures from the apex of ligule downwards, resulting in the senescence of a ligule. In a well developed ligule, the surface of free end is constantly covered by a cuticle layer. Along with the growth of the ligule, the overlapping pattern of cells and the intercellular spaces in neck cell region become more obvious. Consequently the cuticle layer is sloughed off gradually (Figs. 35, 36). The cells of the leaf base outside the sheath become converted into short spiral to reticulated tracheids (Figs. 7-10).

II. Development of ligule

The first ligule primordium is recognized in the adaxial surface of the second leaf primordium (Fig. 1). Two to three superficial cells located at the junction between the young leaf and stem, contributing to the ligule initial cells (Figs. 1, 37). These cells grow larger with their free walls protruded outwards (Fig. 38). Following the enlargement of initial cells, the first division occurs in these cells are periclinal to leaf surface (Fig. 39). The lower derivative are the initial cells of both sheath and glossopodium. After the first periclinal divisions and slight elongation, the lower derivatives divide periclinally again. Their upper daughter cells, larger than their lower ones, continue to elongate and give rise to periclinally arranged glossopodial cells (Figs. 35, 41). Their lower daughter cells divide once in each cell to become the pairing sheath cells of ligule (Figs. 40, 41). On the other hand, the upper derivatives of the superficial initial cell continue to divide variously to give rise to the free protrusion of ligule. The continuous divisions result in that the ligule extends upward from the leaf surface. In subsequent stages, the ligule increases in width and height (Fig. 37). The differentiation of the basal cells and neck cells occurs in this stage. In later stage, some young neck cells cease to divide earlier than the others in traverse face resulting in formation of various depth-lobed appearrance in the free apex of the ligule (Figs. 3-5).

Figs. 37-42. Various developmental stages of ligule.

Fig. 37. Two to three initial cells (arrow) and the adaxial surface of the second leaf primordium (bar=20 μm).

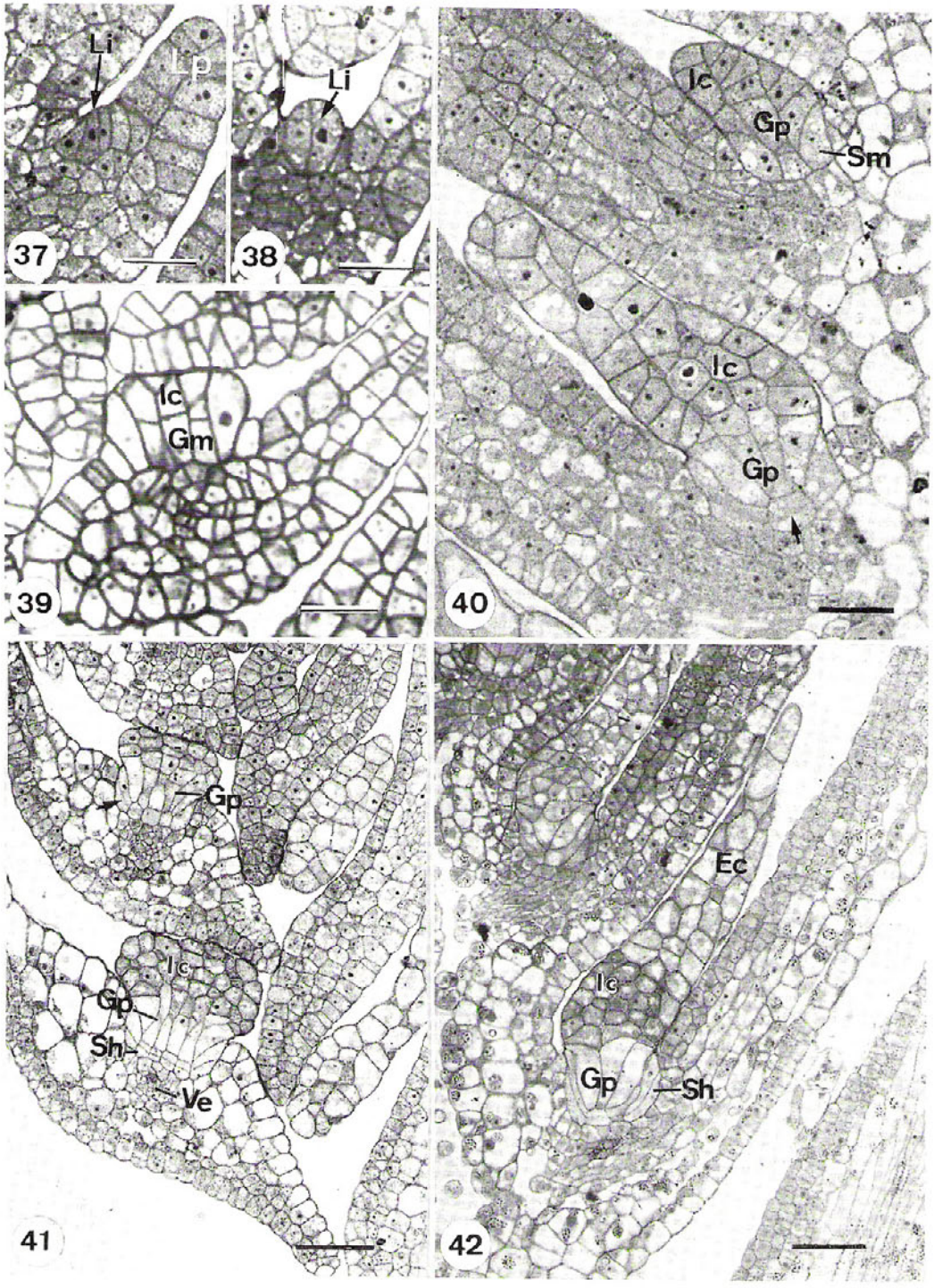
Fig. 38. Initials protruding outwards (bar=15 μm).

Fig. 39. Formation of basal- and glossopodial mother-cells (bar=15 μm).

Fig. 40. Upper ligule—sheath mother cells; Lower ligule—formation of periclinal division (arrow) in some sheath mother cells (bar=15 μm).

Fig. 41. Section through the basal part of ligule, showing the differentiation of sheath and glossopodium (bar=30 μm).

Fig. 42. A well differentiated ligule (bar=30 μm).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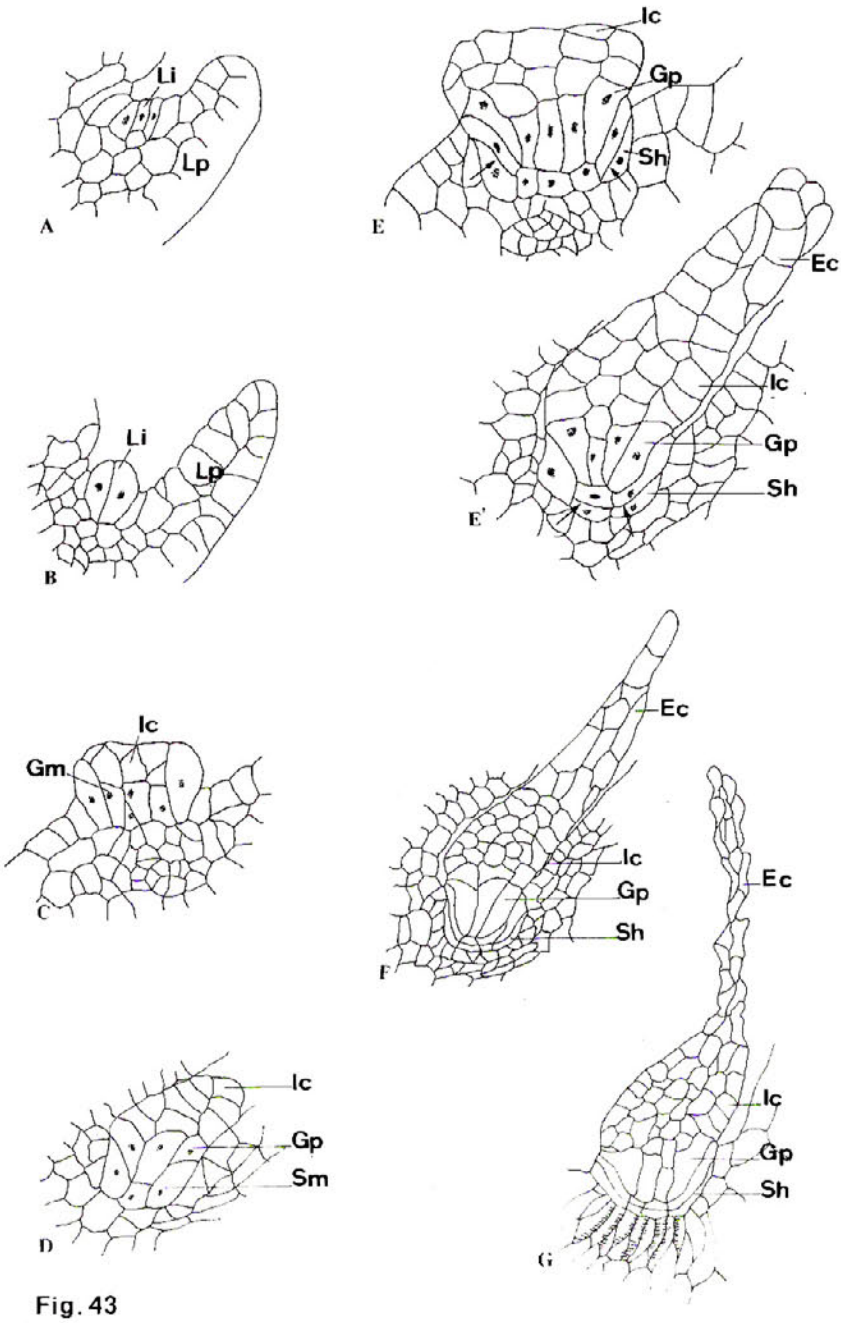

Fig. 43

Fig. 43. Drawings showing the development of ligule, explanation refer to: Fig. A—Fig. 37; B—Fig. 38; C—Fig. 39; D—Fig. 40 upper; E—Fig. 41 upper; E′—Fig. 40 lower; F—Fig. 42; G—Fig. 8.

DISCUSSION

The present study shows that the ligule arises from at least two rows of cells, and that agrees with the case described by Hofmeister (1851) and Gibson (1896), but differs from that reported by Pfeffer (1871) as arising from a single row of cells. These workers took different species for their observation. The initial cells are quite remarkable in micromorphology. Therefore it may be said that the number of the initial cells of ligule in *Selaginella* varies according to the species.

As it is the same in *Isoetes* that the young ligule in this plant is hemi-transparent. It becomes brownish and withered in later stage. No case is the complete separation of ligule from the leaf, although there is a distinctive cellular difference in the ligule sheath and its contiguous cells of leaf tissue. The cell changes during the withering of ligule in *Selaginella* somewhat differ from that in *Isoetes* (Yang, 1973). No cell separation that commonly occurs in *Isoetes* ligule, is seen in the ligule of the present plant, even at the extreme apex of the neck cells.

Though the cell differentiation is detectable as four regions within the ligule in both *Isoetes* and *Selaginella*, it is more distinct at the level of cell size, cell shape and protoplasmic density among four regions in *Selaginella* than that in *Isoetes* (Smith, 1900; Goswami, 1975). In a longitudinal section, four regions of cell differentiation are also recognized in the present observation as described in several earlier reports for some other species (Horner *et al.*, 1975; Smith, 1900). The most interesting and important character described for the cellular structure in these studies is the presence of wall ingrowth on the outer periclinal wall of upper sheath cells, the wall separating upper sheath cells and glossopodial cells (Horner *et al.*, 1975; Pate, 1972). In addition to the outer periclinal wall of upper sheath cells described by above workers, the same structure is also found existing on the lower sheath cells contiguous with leaf cells as well as the basal wall of glossopodial cells in the present species. The wall ingrowth of these cells is very conspicuous in a well developed ligule. In other words, the transfer cell-like structure appears only at certain developmental stages of the ligule. No wall ingrowth is found in the juvenile ligule. Besides, starch-containing plastids are found in both sheath cells and basal cells of young ligule, but none in the mature ones. On the contrary, the tracheids connected the leaf vein and ligule sheath are not formed until the ligule reaches it full size. The intimate interrelations between structure and function are by no means ignored. Various structural changes occur as the ligule develops. Different physiological fates would be suggested for various developmental stages of ligule just as is true of every plant organ. The most important function for the ligule is supposed to be secretion. Some cellular structures which have considered to be closely related with secretion are found in the ligule of the present material. They are the presence of wall ingrowth of both upper and lower sheath cells which are exactly connected with the leaf vein by means of the tracheids in between. The existence of numerous plasmodesmata on the wall separating upper and lower sheath cells, and the wall between glossopodium and the lowest basal cells show that it would provide the continuous phase in regard to the movement of secretory material, or its precusor in leaf vein, sheath cells, glossopodium and basal cells. However the basal cell seems not be merely the region for the movement of some materials. The basal cells are characterized by the evident mitochondria, dictyosomes, dense protoplasm

and the pecullar arrangment of endoplasmic reticulum i. e., running parallel to the nuclear envelope. It is more likely to be suggested as the synthesing region rather than a passage region. Though the exact secretory material has not been detected these prominent morphology of cellular organelles would support that certain activities proceed within them. It may be mucilage as detected in some species of *Selaginella* or water (Bilderback, 1987; Gibson, 1896). Another function, absorption of the ligule has been assumed (Kohlenbach & Geier, 1970; McNab, 1887). Entire free surface of the well developed ligule is fairly covered by cuticle. It forms a barrier to absorption, at least for the mature ligule. The direct release of substance from the ligule seems not be the case because of the thick cuticle which bears no pores. The evidence of the present study suggests that the secretory substance probably remains in the intercellular spaces located among the neck cells formed at the later stages. It may be released after the cells break down at the time of ligule senescence. The same thinking has been also presented by earlier workers (Horner, *et al.*, 1975; Sigee, 1974). The present results are not able to reveal further information on the function of ligule, but the finding on the widely existence of transfer cell-like structure at the basal part of ligule should not be ignored in explaining the function of the ligule.

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全緣卷柏之葉舌

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摘　要

本文探討全緣卷柏 [*Selaginella delicatula* (Desv.) Alston] 葉舌之解剖和發生過程。葉舌之外形由單一指狀、至二、至多裂瓣狀之掌狀都有。組成葉舌之細胞明顯地可分爲下列四組織區：舌鞘、舌足、基部和頸部。舌鞘由雙層之扁形細胞組成，舌足則爲單層之細長且高度液胞化之細胞，一成長的葉舌之舌鞘及舌足細胞內皆具有內凸之胞壁，卽爲類似轉送細胞之構造。基部細胞之胞質濃、形等徑，而頸部之細胞則爲長條形之細胞相互交錯而排。至後期，葉舌雖不脫落，但漸萎縮而老化。頸部最先呈萎縮狀。本文並根據細胞形態推測葉舌內各組織區之可能功用。

葉舌起源於二至三列之葉原原始表層之細胞，最早分化者爲基部細胞，其次爲舌足細胞，再次爲舌鞘細胞，最後爲頸部細胞。