MORPHOLOGY AND DEVELOPMENT OF BULLIFORM CELLS IN *ARUNDO FORMOSANA* HACK.

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Abstract: Bulliform cells of *Arundo formosana* Hack. occur intercostally in adaxidal epidermis of leaf blade. They are the most vaculoated among all cell types in epidermis. They contain very few organelles. Builiform cell initiates in protoderm that is located in the sinus between two procambia. The site and initiation sequence of bulliform cells are closely related with the formation of procabium. The later enlargement of bulliform cell occurs synchronously, and is limited by the presence of subepidermal sclerenchyma neighboring it. The enlargement of bulliform cells would play an important role in expansion of the developing blade.

INTRODUCTION

The bulliform cells which are large, thin-walled and highly vacuolated cells occur in all monocotyledonous orders except the Helobiae (Linsbauer, 1930; Metcalfe, 1960). In the grass leaf, the bulliform cells generally occur in intercostal row of several cells in width. Sometimes, several enlarged mesophyllous cells, colorless cells, are located subjacent to the bulliform cells (Metcalfe, 1960). The morphology of bulliform cells combined with colorless cells have been used as the taxonomic characters (Metcalfe, 1960). A number of functions have been assigned to the bulliform cells. Haberlandt (1914) have believed that hygroscopic tugor changes of the bulliform cells, also termed motor cells, could cause surface-reducing movements in mature xeric leaf. Burström (1942) found that bulliform cells were not actively or specifically concerned with unfolding and hygrosocopic movement in wheat leaf. Prat (1948) have also described bulliform cells as 'aquiferous, strongly turgescent cells, sometimes reaching a great volume by expanding perpendicularly to the leaf surface'. Shields (1951) have studied on the leaves of some xeric grasses, and observed that the bulliform cells would not involved in involution mechanism. On the contrary, he suggested that subepidermal sclerenchyma and other adaxial elements of mesophyll rather than bulliform cells contributed to involution. Metcalfe (1960) reported that the leaves of *Ammophila arenaria* (Marram Grass) could be tightly rolled under water stress, but the bulliform cells appeared to be less developed.

Esau (1977) suggested that during excessive loss of water, the bulliform cells, or in conjunction with colorless cells, became flaccid and enabled the leaf to fold or to roll. Ellis (1976) have accurately discussed on the literature refers the bulliform cells, and suggested with caution in assigning them a role in leaf movement. However, Linsbauer (1930) have suggested that water storage was the sole purpose of bulliform cells.

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The purpose of this report is to study the morphology and development of the bulliform cells in *Arundo formosana* Hack. Bulliform cells are the very distinctive members in the majority of grasses including *Arundo formosana* Hack. One probably could find the significance of bulliform cells by study of developmental pattern which has been little studied.

MATERIALS AND METHODS

Arundo formosana Hack. was collected from field. The well expanded leaves were cut at 4 mm long 1 mm wide, and the immature rolled leaves were cut at 4 mm long. Specimens were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer for 4-8 hr at room temperature. After three 20 min buffer rinses, materials were postfixed in 0.1% OsO_4 in the same buffer for 4 hr at room temperature, then rised three times in buffer, 20 min each. Dehydration was completed by an acetone series and Spurr's resin was used for embedding (Spurr, 1969). Sectioning was done with Ultracut E ultramicrotome. For light microscopy the 1 μm thick sections were stained with 0.5% toludine blue. The thin sections were stained with 6% uranyl acetate and lead citrate (Reynolds, 1963), and a Hitachi-6000 TEM was used for viewing. For scanning electron microscopy (SEM), dehyration was done by an alcohol-acetone series, then critical point dried and coated, and viewed with a Hitachi S-520 SEM.

RESULTS

I. Morphology of bulliform cell

The bulliform cells occur intercostally in group in the adaxial epidermis of leaf blade (Fig. 1). Each bulliform cell group exhibits 3-7 cells as seen in cross section. The bulliform cells are so distinct that can be easily discerned from their neighboring long epidermal cells (Figs. 2, 3). The cell size in a single group appears to be alike, or varies fairly. According to the cell size and pattern of arrangement, the bulliform cell group can be identified as three combinational types. Type I is made up of one central large cell and 2-4 smaller neighboring cells (Figs. 2, 4). Type II is two central large cells with 2-4 smaller neighboring cells (Figs. 3, 5). Type III is three or more central large cells sorrounded by none to four smaller neighboring cells (Fig. 6). Since a series of identical bulliform cells are connected one after anther arranging in parallel with the veins, the cell morphology does not show much variation along the entire combinational group from the base to the tip of leaf blade (Figs. 8, 9). The distributional pattern of these combinational types in the adaxial epidermis is at random except the type III (Fig. 7). It always occurs near the leaf margin.

Bulliform cell is the largest cell among the epidermis and its outer periclinal walls shows thicker than the other faces (Fig. 10). Bulliform cell is the most vacuolated among all cell types in leaf. Its cytoplasm occupies very small proportion and is pushed to cell periphery by a large vacuole. It contains a flat nucleus and a few mitochondria, endoplasmic reticula and plastids (Figs. 11, 12). The mitochondria have more inclusions than other organelles. The plastids contain plastoglobuli and are thylakoid-less. They are different from the plastids of long cells. The plasdids of long cells contain plastglobuli and thylakoids, but the thylakoids do not form typical grana (Fig. 13). The cell walls connected

Key to labelling:

BC: Bulliform cell; CC: Colorless cell; Er: Endoplasmic reticulum; F: Fiber; LC: Long cell; Mi: Mitochondrion; MV: Midvein; N: Nucleus; P: Plastid; Pg: Plastoglobuli Pl: Plasmodesma; Th: Thylakoid; V: Vein.

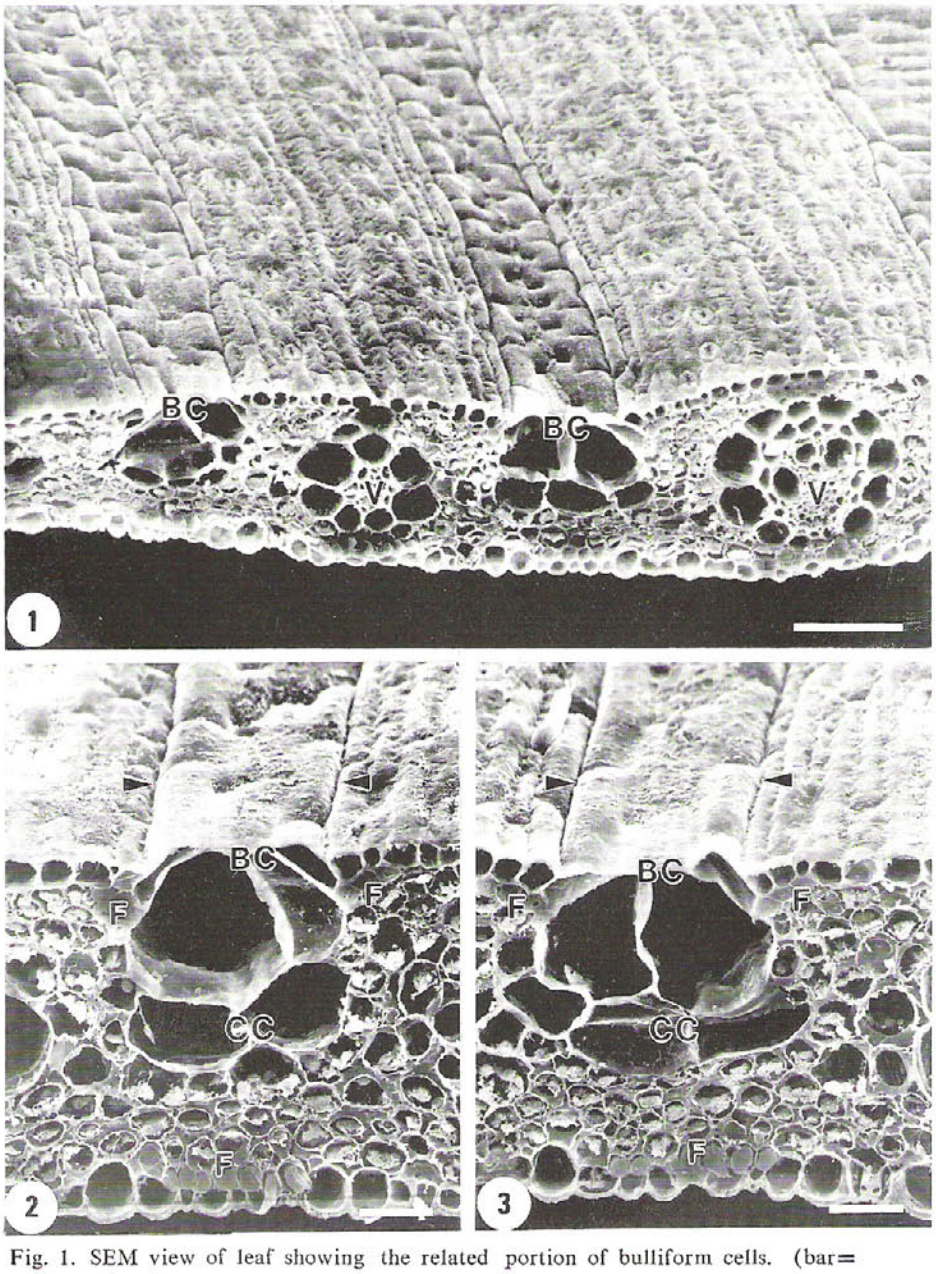


Fig. 1. SEM view of leaf showing the related portion of bulliform cells. (bar= 50 μm)

Figs. 2, 3. SEM view of leaf showing the cellular distinction (arrowhead) between bulliform cells and long cells. (bar=15 μm)

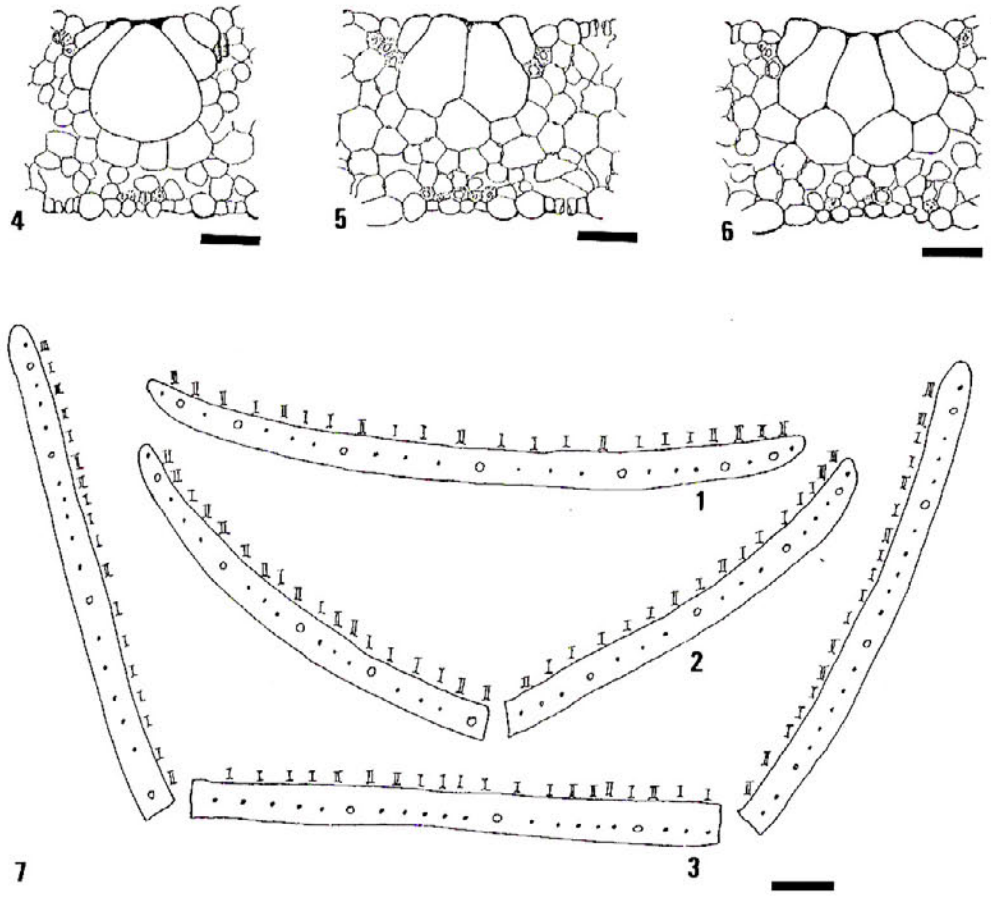
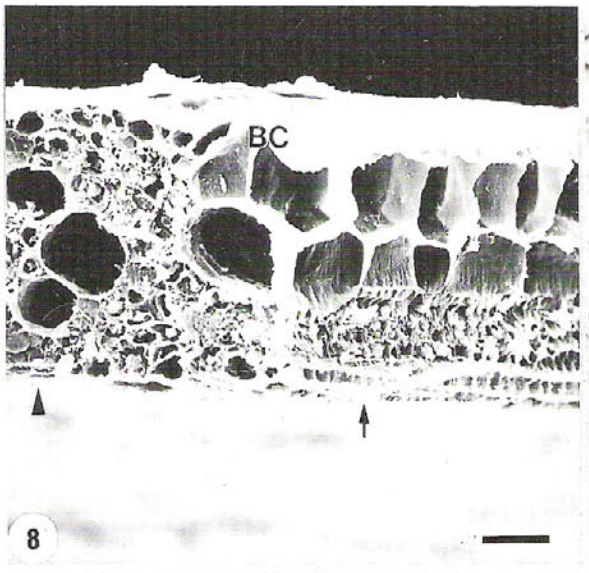


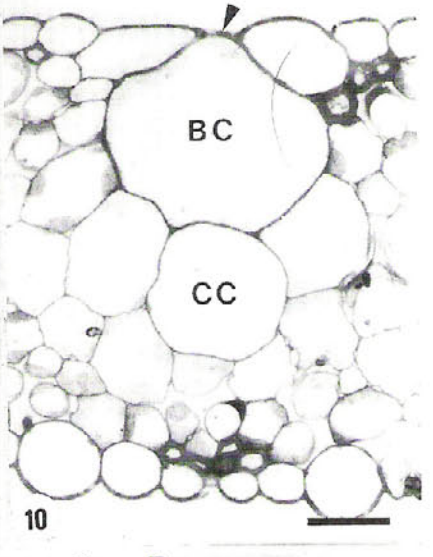
Fig. 4–7. The drawings of leaf blade in cross section.
Fig. 4. Combination type I of bulliform cells. (bar=20 μm)
Fig. 5. Combination type II of bulliform cells. (bar=20 μm)
Fig. 6. Combination type III of bulliform cells. (bar=20 μm)
Fig. 7. The distributional pattern of three combination types on leaf blade. (○—large vein, ●—small vein. 1, 2, 3 are three representative leaf blades of different size). (bar=0.1 cm)

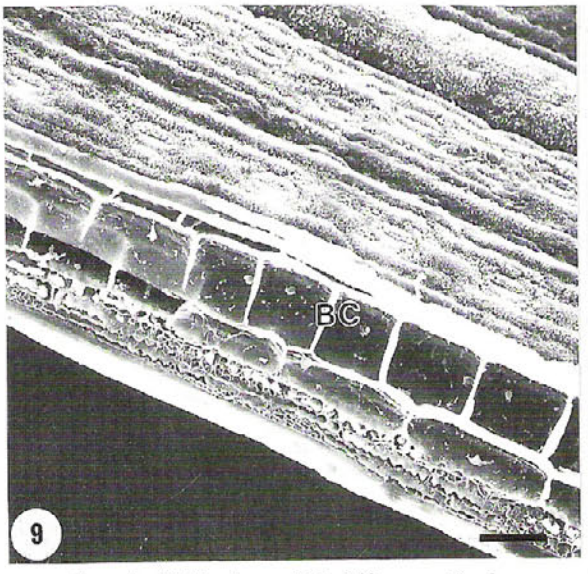
bulliform cell and its neighboring long cell appear to very thin and bears many pits with conspicuous plasmodesmata (Fig. 14). The cell walls located between the bulliform cells and colorless cell is also thin, but only bears a few pits. The cell wall separates two bulliform cells is the thickest and bears fewer pits (Fig. 15).

II. Development of bulliform cell

The leaf bears 7 to 12 larger veins including midvein, and 1 to 5 small veins in each space between two large veins (Fig. 7). Like in other grasses, all veins are oriented longitudinally, converge and join at the leaf apex. The midvein is the first to differentiate (Fig. 16). The rest of veins initiate during the lateral growth of leaf blade. As seen in cross section, the first pair of lateral large veins form







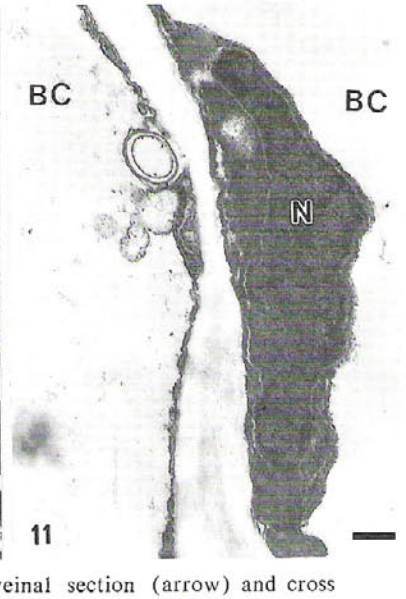
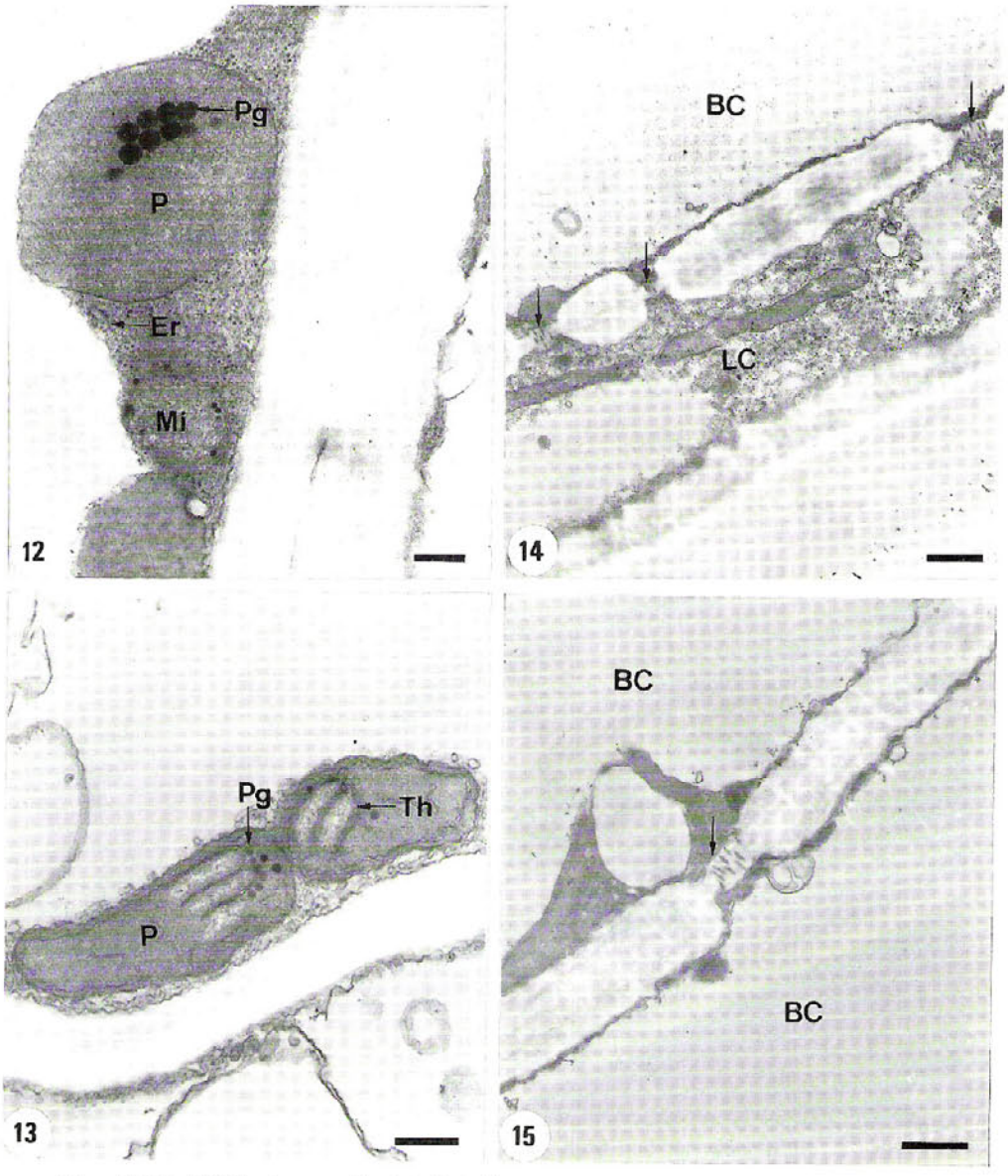


Fig. 8. SEM view of bulliform cells in paraveinal section (arrow) and cross section of a vein (arrowhead). (bar=25 μm)
Fig. 9. SEM view of bulliform cells in paraveinal section. (bar=25 μm)
Fig. 10. Transverse section of leaf showing thicker outer periclinal wall (arrowhead) of bulliform cell. (bar=15 μm)
Fig. 11. Ultrastructure of bulliform cell showing nucleus. (bar=1 μm)

at both sides of midvein (Figs. 16-18). Then the subsequent large veins appear between the midvein and lateral large veins (Figs. 18-20). The small veins originate after its contiguous large veins which are at the procambial stage, and located between two large veins (Fig. 21).

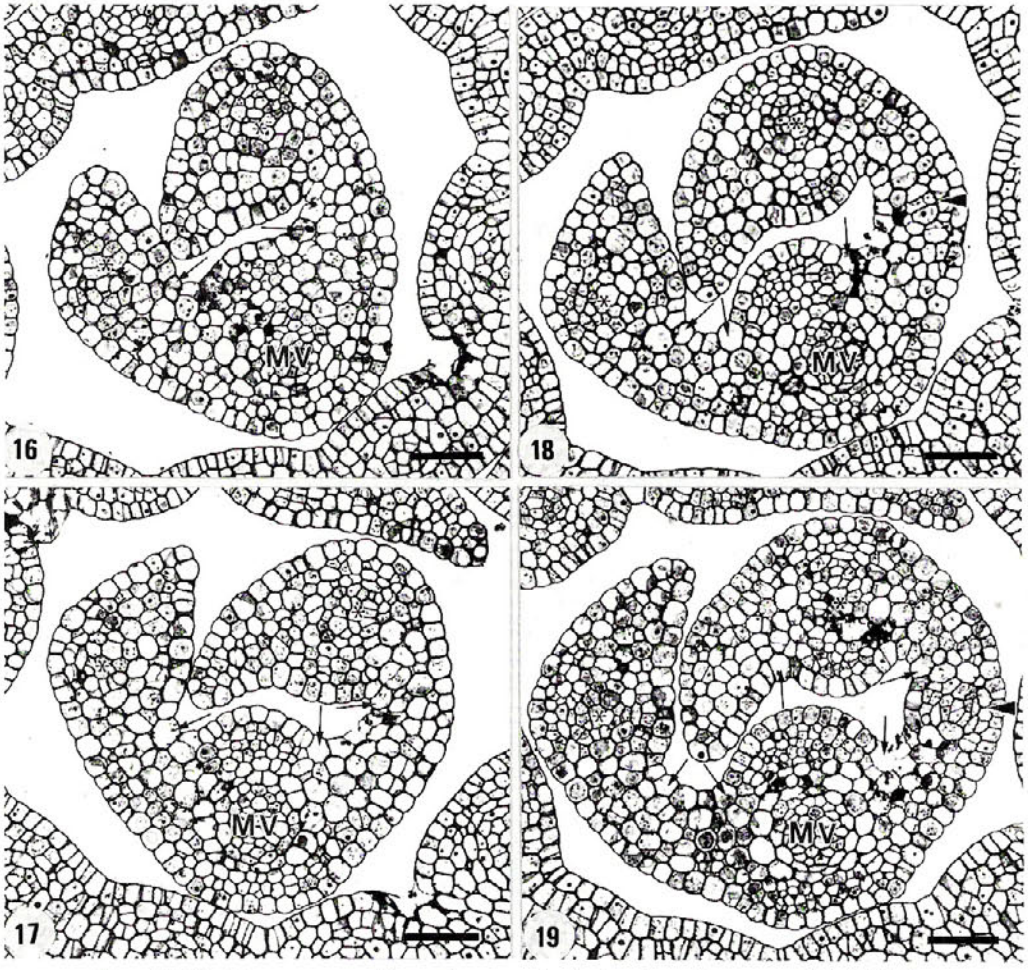
The sequence of the initiation of bulliform cell group in leaf blade is closely related with its associated vascular strand rather than its contiguous bulliform



Figs. 12-15. TEM micrographs showing ultrastructure.
Fig. 12. Plastid, mitochondria and endoplasmic reticulum in bulliform cell. (bar=1 μm)
Fig. 13. Plastids in long cell. (bar=1 μm)
Fig. 14. Long cell, note the pits (arrow) on the wall next to bulliform cell. (bar=2.5 μm)
Fig. 15. Bulliform cell wall, note the pit (arrow). (bar=2.5 μm)

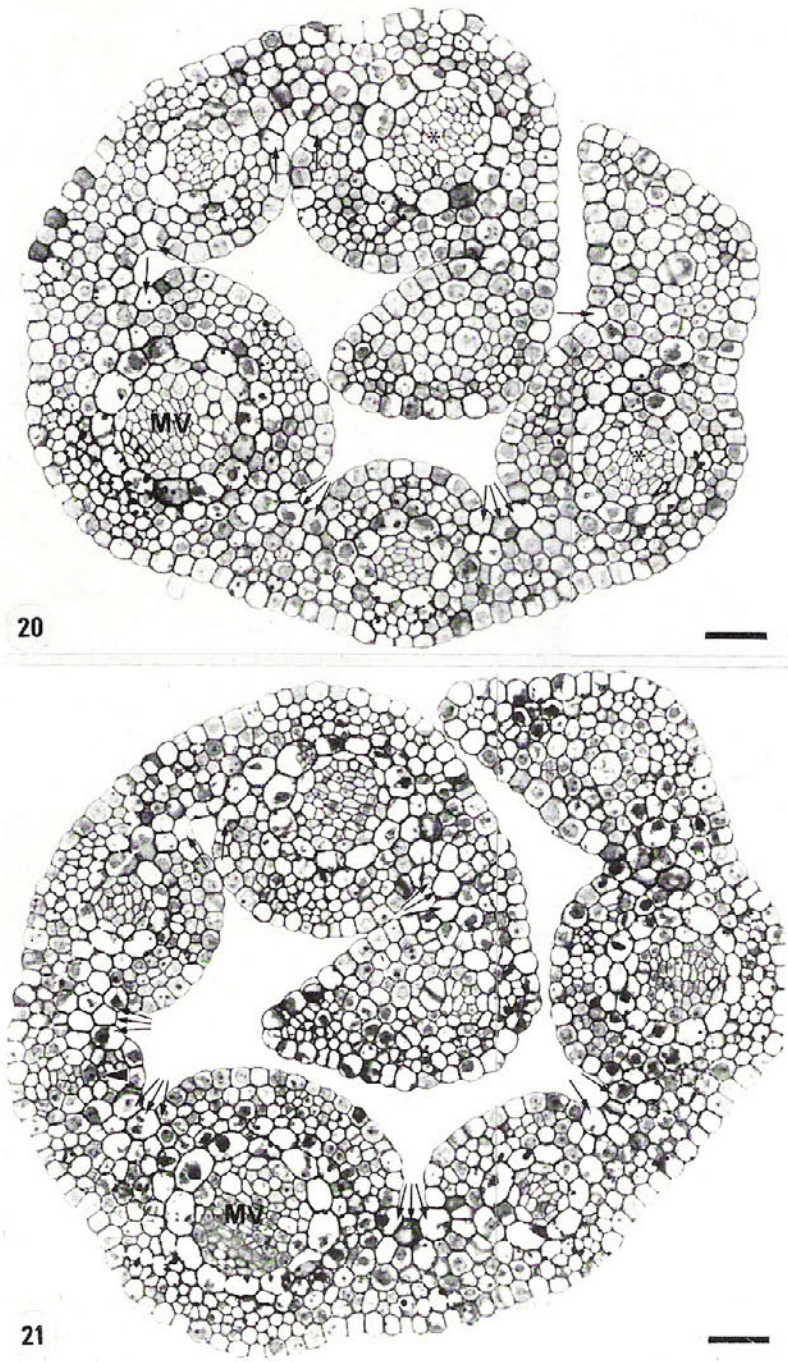
cell group. The bulliform cell originates very early during the leaf formation. However the procambium initiates slightly ealier than its associated bulliform cell group (Fig. 16).

The first indication of the initiation of bulliform cell is the cell enlargement.

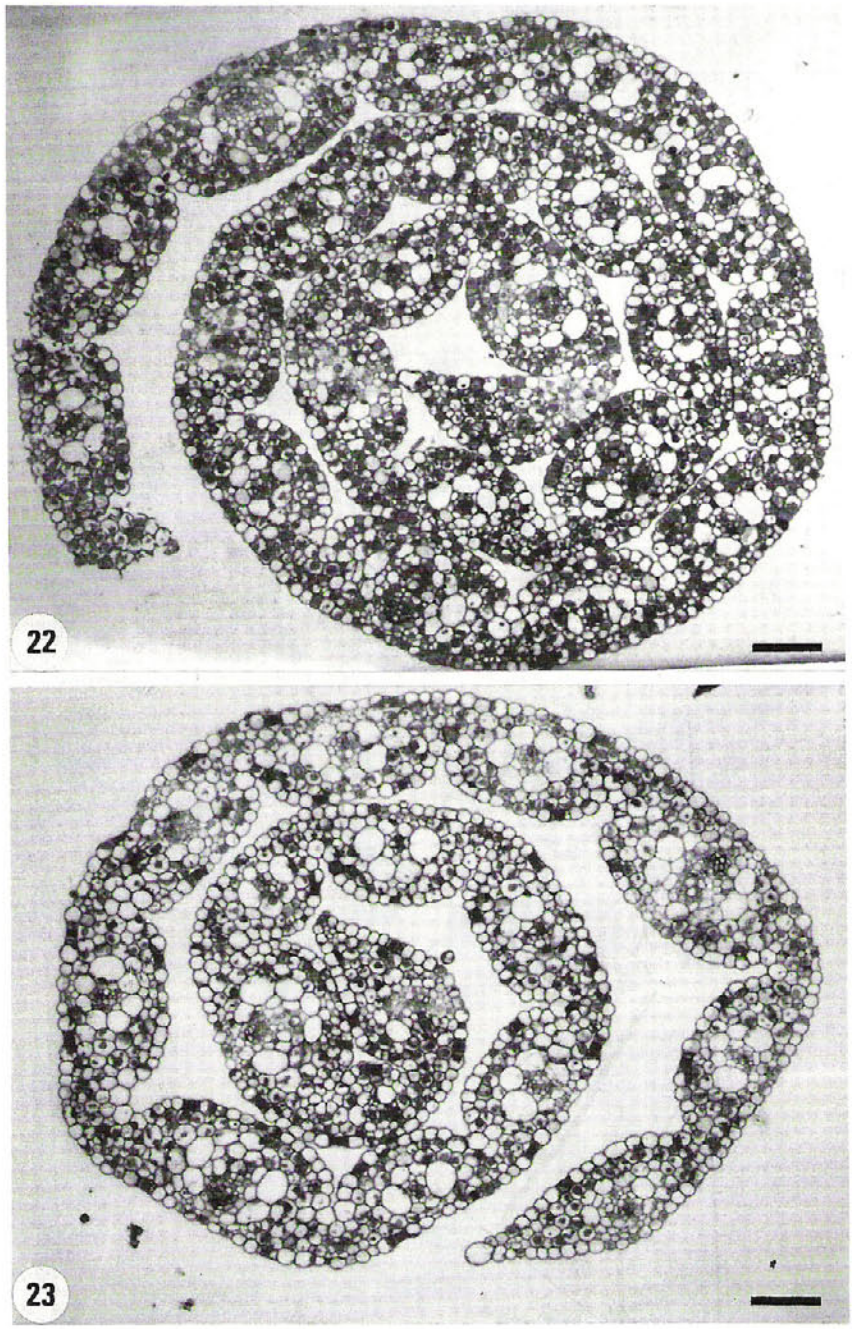


Figs. 16-19. Transverse sections of young leaf showing the sequential initiation of bulliform cell, bulliform cell initial (arrow), lateral large vein (*) and large vein (arrowhead) formation. (bar=30 μm)

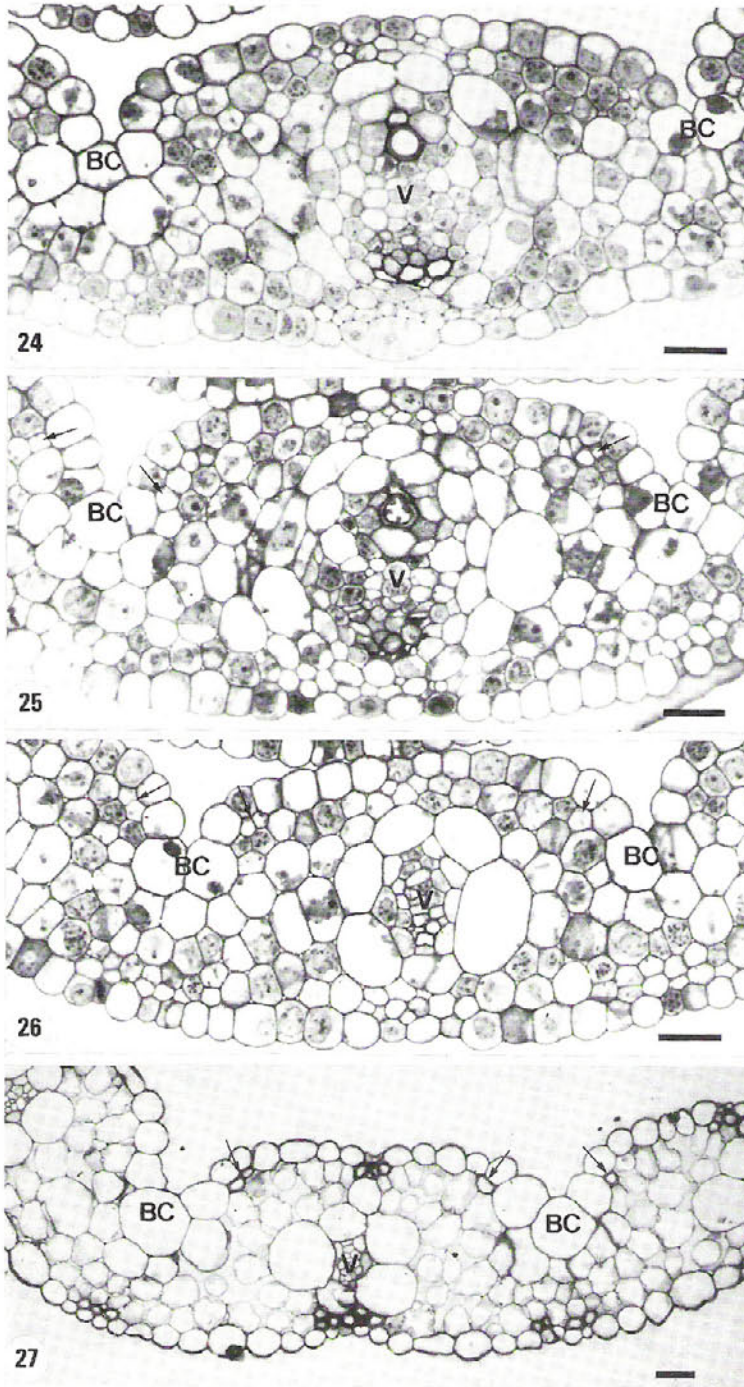
The initial group of bulliform cells start to differentiate at the stage when the procambial strands are counted as three (Fig. 16). The first pair of initial group of bulliform cells form in adaxial epidermis on both lateral sides of young midvein (Figs. 16-19). The young leaf is still rolied. The subsequent bulliform cell initials form at the intercostal regions slightly after after their associated procambia appear (Figs. 16-21). Apparently, the sequence of the initiation of bulliform cells keep pace with the procambium in early ontogeny. However, the successive enlargement of bulliform cell does not occur until all bulliform cell groups in the entire leaf blade complete their earliest cell enlargement. So that all the bulliform cell group remain as almost the same developmental stage at the time of the formation of the last procambium near the leaf margin. As soon as all juvenile bulliform cell groups in the entire leaf blade appear, the subsequent enlargement starts almost sychronously (Figs. 22, 23). Consequently the size of cell and the degree of growth in all the members shown in the same cross section exhibit



Figs. 20, 21. Transverse sections of young leaves showing a series of sequential development of bulliform cells and procambia, bulliform cell initial (single arrow), developing bulliform cells (triple arrow) and small vein (arrowhead). (bar=30 μm)



Figs. 22, 23. Transverse sections of young leaves (older than in Figs. 20, 21) showing the intercostally developing bulliform cell groups in the adaxial epidermis. (bar=50 μm)



Figs. 24-27. Transverse sections of young leaf showing the different stages of bulliform cell enlargement and the sclerification of subepidermal (arrow). (bar=20 μm)

alike (Figs. 22, 23). The onset of synchronous dominant enlargement in bulliform cells occur slightly prior to the maturation of last vascular elements, i. e., metaxylem (Figs. 24-27).

One to several strands of fiber cells are constantly present in the subepidermis adjoining the bulliform cells in a rather mature leaf blade (Figs. 2, 3). The sclerification of these subepidermal fibers proceeds during the later enlargement period of bulliform cell. The bulliform cells expand homogenously in all directions before the subepidermal fiber begins to sclerify (Fig. 24). As the subepidermal fiber become sclerified, the expansion process is limited on the face next to fiber. Consequently the bulliform cells grow larger with their walls inwards mesophyll (Fig. 25-27). Thus, majority of the well developed bulliform cells exhibit funnel-shape in sectional view (Figs. 2, 3).

In the further stage, the enlargement of both bulliform cells and their peripheral colorless cells would cause the expansion of rolled leaf blade, since the outward-enlargement of bulliform cells and colorless cells take place (Fig. 27). Finally the sinus region where the young bulliform cells located disappear and the bulliform cells become even level with other epidermal cells, resulting in the spread of entire leaf blade.

DISCUSSION

This observation on the morphology of bulliform cells of *Arundo formosana* is in general agreement with those of many grasses (Kuoh, 1985; Metcalfe, 1960). As described by Kuoh (1985), the bulliform cell group in the present species is constantly present in adaxial epidermis between the vein ribs. It is grouped as *Arundo* type by Metcalfe (1960). However the present examination demonstrates some variations occuring for the species even the same leaf. Three different types of bulliform cell groups are recognized based on the size and arrangement of individual cell within the bulliform cell group (Fig. 7). One of these three types occurs near the leaf margin only whereas the other two are randomly distributed.

The pattern of wall thickening in bulliform cell is the same as in the majority of descriptions for this element (Esau, 1965). In this study pits with conspicuous plasmodesmata were seen on all the faces of bulliform cell wall. The wall separating two bulliform cells bears the fewest pits and appears to be thicker than that of other faces. This fact indicates that it would provides the fast movement of water to enter or leave the bulliform cell from the cells other than its contiguous bulliform cells. The cell lumen is almost occupied by a large vacuole. In comparison with that of long cell in epidermis, the cytoplasmic organelles are scanty, but no significant differences in their basic structure were observed except the plastids. The rather well developed thylakoid was found in long cell, but not in the bulliform cell. These observations do indicate that some cytological differences as well as some peculiar functions were established in the bulliform cell.

Most of the workers on the bulliform cell have suggested that the bulliform cell is relevant to the water movement as we think (Esau, 1977; Haberlandt, 1914; Linsbauer, 1930). We have checked the rolling mature leaf in which the bulliform cells together with their adjacent colorless cells show the serious shrinkage, but not the other epidermal cells. As a matter of fact, the shrinkage of any adaxial epidermal cell would cause the rolling and folding of leaf blade. On the contrary, the cell expansion would contribute to the spread of leaf. It is apparent that the

bulliform cells together with their contiguous colorless cells do take part in the hygroscopic movement in mature leaf.

The study on the initiation and development of bulliform cell have received less attention than that on the morphology and function (Burstöm, 1942; Esau, 1965, 1976; Haberlandt, 1914; Linsbauer, 1930; Metcalfe, 1960; Schields, 1951). The initiation of bulliform cell intimately keeps pace with the formation of procambial strand located in the closest vicinity of it. The most interesting aspect in the development of bulliform cell appears to be the synchronous enlargement of bulliform cells in the later stage of development. Esau (1977) has mentioned that the rolling leaves emerged from the bud is a growth phenomenon based on the enlargement of bulliform cells and mesophyllous cells. The evidence present here shows the similar growth pattern as Esau's observation (1977). The synchronous enlargement of bulliform cells in the developing leaf of *Arundo formosana* results in the even lateral expansion and spread of leaf. Besides, the presence of hypodermal sclerenchyma enforces the unilateral growth of bulliform cells.

LITERATURE CITED

BURSTRÖM, H. 1942. Über die Entfaltung und Einrollen eines mesophilen Grassblattes. Bot. Notiser. 1942: 351-362. Cited by Esau (1965) p. 153.

Ellis, R.P. 1976. A procedure for standarding comparative leaf anatomy in the Poaceae. I. The leaf-blade as viewed in transverse section. Bothalia **12**: 65-109.

ESAU, K. 1965. Plant anatomy. 2nd ed. John Wiley & Sons, Inc.

ESAU, K. 1977. Anatomy of seed plant. 2nd ed. John Wiley & Sons, Inc.

HABERLANDT, G. 1914. Physiological plant anatomy. p. 599. London: Macmillan & Co.

LINSBAUER, K. 1930. Die Epidermis. In: Handbuch der Pflanzenanatomie. K. Linsbauer, ed. Band 4. Lief. 27.

KUOH, C.S. 1985. The comparative leaf anatomy of the Poaceae in Taiwan, with special reference to the Kranz syndrome. Ph.D. dissertation, Department of Botany, National Taiwan University, R.O.C.

METCALFE, C.R. 1960. Anatomy of monocotyledons. I. Gramineae. Clarendon Press, Oxford.

PRAT, K. 1948. General features of the epidermis in *Zea mays*. Ann. Mo. Bot. Gdn. **35**: 341-351.

REYNOLDS, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**: 208-212.

SHIELDS, L.M. 1951. The involution mechanism in leaf of certain xeric grasses. Phytomorphology **1**: 225-241.

SPURR, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultra. Rev. **26**: 31-43.

臺灣蘆竹泡狀細胞之形態與發育

簡萬能 江蔡淑華

摘 要

臺灣蘆竹之泡狀細胞數個成羣，排列在葉片兩脈之間的上表皮。在表皮細胞中，泡狀細胞爲液胞化程度最甚者，內部胞器極少。泡狀細胞源於兩原原始形成層間凹陷處之原始表層細胞，其起源順序與原始形成層之形成有關。泡狀細胞之後期膨大同步進行，其方向受其兩側之厚壁細胞形成的影響，而泡狀細胞膨大對幼葉片之開展扮演一個重要的角色。