Light and Electron Microscopic Characterization of Husk from Korean Rice

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ABSTRACT

Microscopic techniques were used to observe the microstructure of rice husk. Microscopic examination showed that two main components of husk, lemma and palea consisted of outer epidermis, layers of fibers, vascular bundles, parenchyma cells, and inner epidermis, in sequence from the outer to the inner surface. The outer epidermal walls were extremely thick, highly convoluted and lignified. The underlying fibers were also thick-walled and lignified. Parenchyma cells were thin-walled and unlignified. Inner epidermal cells were also unlignified. The outer surface of both lemma and palea were conspicuously ridged, but the lower surface had a flat appearance. As part of a detailed study to characterize rice husk using microscopic and micro-analytical techniques, distribution of silica was also examined, and is presented elsewhere. Rice husk can potentially be used as a raw material for making composite products and the observations presented here form valuable background information for our future work related to product development.

Key Words : rice husk, lemma, palea, light microscopy, scanning electron microscopy, transmission electron microscopy

INTRODUCTION

The use of silica from rice husks in the manufacture of high value materials has attracted much attention and recently been reviewed (Sung and Gong 2001). Interest in the use of entire rice husk for the manufacture of composite products is also growing. The use of rice husk in making composite products such as fiberboard and lignocellulosic fiber-thermoplastic composites is also attracting much attention because of the potential for enormous gains in certain important properties of these products (English et al., 1997; Ismail et al., 2001). The performance of composite products is determined by the structural, chemical, physical, and engineering properties of their individual components, and in the case of rice husk its fragments and fibers. Chemical characterization of and silica distribution in rice husk from Korean rice species have been characterized well (Chung et al., 1998). A brief description of the anatomy

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of rice husk is also available(Matsuo and Hoshikawa, 1993). However, detailed information on the organization and properties of internal tissues of rice husk is vital for optimizing processes and enhancing properties of the composite products made from this important natural ligno-cellulosic material. We have characterized the surface features and internal organization of this material in detail with these considerations in mind.

MATERIAL AND METHODS

The rice husk from Korean rice plant species, which was obtained from a local rice milling plant was washed to remove some rice particles and other debris. The rice husk was air dried before being used for microscopic examination. For observation with scanning electron microscope (SEM), and field-emission SEM (FE-SEM), dried rice husk was soaked in distilled water, critical-point dried, and then coated with gold in a sputter coater. The samples were observed with a SEM (Hitachi S2400) and FE-SEM (Hitachi 4700 at the Korea Basic Science Institute, Gwangju) at an acceleration voltage of 15 kV and 5 kV, respectively. For light microscopy (LM) and transmission electron microscopy (TEM), rice husks were dehydrated in acetone and embedded in Spurr' s low viscosity resin.

For LM, transverse sections were cut at 2~3 ㎛ thickness with a RMC MTX ultramicrotome using glass knives. The sections were either examined unstained (under polarized light) or after staining with 0.05 % toluidine blue (prepared in 1.5% borax) with a Zeiss Axiolab photomicroscope. Husks were also hand-sectioned with a razor blade, and the sections were stained with phloroglucinol-HCL prior to examination with the photomicroscope.

For TEM, transverse ultrathin sections were cut with the same microtome using a diamond knife. Sections were stained with 1% $KMnO_4$ (prepared in 0.1% w/v sodium citrate) for 5 minutes at room temperature and then examined with a JEOL 1010 TEM.

RESULTS AND DISCUSSION

The main components of rice husk are lemma and palea, which interlock with each other at their margins to provide a tight seal and also protection for the enclosed developing grain. These two components are similar in certain aspects of their morphology and microsturcture but differ in other characteristics. The surface morphology of lemma and palea was similar, and therefore the surface features of lemma only are described here. Similarly, TEM information is provided only for lemma. However, internal tissue organization is described for both lemma and palea, revealing significance differences.

SEM views of surface features and internal tissue organization of husk are provided in Figs. 1-6. The outer surface of lemma is highly ridged, and the ridged structures have a linear profile (Fig. 1). As seen at high magnifications (Fig. 2), epidermal cells of lemma are arranged in linear ridges and furrows, and the ridges are punctuated with prominent conical protrusions. The outer surface of lemma also contains papillae and hairs of varying sizes, but they were often broken at their bases in the material examined, and therefore are not illustrated. The surface details of inner epidermis are provided in Figs. 3 and 4. Unlike the outer epidermis the cells of the inner epidermis are positioned in the same plane. However, the cells of the inner epidermis are also arranged in parallel rows, and their walls are slightly sinuous (Fig. 3). The inner surface of lemma also contains hairs, which are often broken at their bases (Fig. 3). Stomata are absent in the outer epidermis but are present in the inner epidermis (Fig. 4).

Cross sections taken through the entire thickness of the husk provide information on its internal tissue organization. A low magnification SEM view of an

entire cross section of lemma is shown in Fig. 5. The outer epidermis appears to be highly undulated due to the presence of regularly spaced protrusions. The margins of lemma are intricately folded inwards. Fig. 6 provides high magnification SEM view of part of a cross section of lemma, which includes a vascular bundle. The outer epidermis is extremely thick-walled. The lower epidermis is not well defined because of collapse. Among the internal tissues, the vascular bundle region is well defined. Xylem and phloem tissues are clearly distinguishable. They are enclosed by a bundle sheath.

LM views of toluidine blue stained cross sections of lemma are provided in Figs. 7-11. Toluidine blue is a polychromatic stain, which has been widely used to differentiate lignified tissues from non-lignified tissues. Lignified cell walls generally stain greenish-blue and those which are unlignified stain bluish in color. Phloroglucinol-HCl is known to stain lignified cell walls orangish-pink. Unlignified cell walls are not stained with this stain. Lemma contains three vascular bundles across its width, one of which is centrally located and the other two are positioned near the margin on either side of the central bundle. The central and one peripheral bundle are included in the low magnification view of lemma section shown in Fig. 7. The lemma regions containing vascular bundles are somewhat distended as compared to other regions. Various regions of lemma cross section shown at high magnifications in Figs. 8-11 provide detailed information on internal tissue organization. Fig. 8 illustrates the central and adjoining regions of lemma. The outer epidermis is much contorted and consists of highly thick-walled cells. The inner epidermis shows signs of collapse, and thus the cells in this layer are not clearly defined. The internal tissue is differentiated into outer layers of thick-walled fibers and inner thin-walled parenchyma cells. The central region of lemma, which contains a vascular bundle is slightly distended. Lemma progressively tapers off in its thickness away from the center. In the thinnest regions lemma appears to lack parenchyma cells, and the internal tissue consists only of fibers. Fig. 9 shows a peripheral region of lemma. The extremities of the margins of lemma which curl inward, are somewhat specialized and in some respects different from the remainder of lemma. However, the tissue organization is basically similar to that of other parts, such as the region shown in Fig. 8. The peripheral region of lemma is distinctly thicker than the regions flanking the central vascular bundle, primarily because of the presence of larger numbers of parenchyma cells. The micrographs shown in Figs. 10 and 11 were produced from thinner sections, and thus, because of improved resolution and more controlled staining of sections certain features are better defined. In Fig. 10 the outer epidermal cells consist of extremely thick walls, which enclose a relatively small lumen. The cell walls are also highly convoluted, and this may be another factor why in some cells the lumen is not visible or appears extremely small. The outer epidermal walls are greenish-blue in color, except in parts of the outer tangential wall. The underlying fiber cells, which appear greenish-blue, are small and arranged in two layers, except at the margin of lemma. Here, in places they are arranged in three layers, becoming progressively smaller towards the extremity of the margin. The walls of parenchyma cells are bluish in color. The lower epidermis is also bluish in color and shows signs of collapse. The curled region of lemma margin has a unique structure. Typical outer epidermal cells become smaller, appearing fiber-like in the curled region of the margin. Then, there is more or less an abrupt change, and the outer epidermis of the highly curved region consists of cells which appear similar to the cells of inner epidermis in the thickness and staining of their walls. Internally, this part of lemma margin consists only of extremely small fibers. Fig. 11 shows a region associated with the peripheral vascular bundle.

The outer epidermis is clearly differentiated into dome-shaped cells and less curved (more or less flattened) cells. The lumina of some epidermal cells stain pink. The underlying fibers are arranged in two layers, with some variability in places. The part of the fiber layer overlying the vascular bundle is four cells deep, and the fibers in this region are also smaller. The remaining part of the lemma cross section is filled with thin-walled parenchyma cells. The vascular bundle in this region appears to be smaller than that in the central region of lemma. The cells of inner epidermis are collapsed.

LM views of toluidine blue stained cross sections of palea are provided in Figs. 12-14. Fig. 12 is a low magnification view of a cross section through palea. Although tissue organization of palea is similar to that of lemma, distinct differences occur in the location of peripheral vascular bundles and the shape and organization of the margins. The palea, like lemma, also contains three vascular bundles, one central and two peripheral, but as shown in higher magnification views, the location of peripheral bundle is somewhat different as compared to that in lemma. The hook at the extremity of the margin of palea forms an intimate contact with the curled region of the lemma by hooking onto it, and thus forming a tight seal. A cross section through the central region of palea is illustrated in Fig. 13. The tissue organization is similar to that of the corresponding region of lemma with minor exceptions. The central region of palea appears to be thicker than lemma, primarily because of the presence of larger parenchyma cells, which are also more abundant in this region of palea as compared to lemma. The central vascular bundle of palea is also more prominent and the bundle sheath is more distinct. The features of outer epidermis, inner epidermis and fiber layers share similarities with lemma. Fig. 14 shows a cross section through the peripheral part of palea, which is thinner than the central part and terminates into a hook at the margin, with a complex, intricate tissue organization. The tissue organization at the margin is very different from that in the adjoining peripheral region, which consists of two layers of fibers sandwiched between outer and inner epidermal layers. The margin consists of a prominent hook region, which terminates into a slender filamentous structure. The hook of palea, in contrast to the marginal curl of lemma, curves towards the outer epidermis. The hook region contains a vascular bundle, which is positioned more or less opposite the tip of the hook, and is associated with abundant thin-walled parenchyma cells. These tissues are sandwiched between the outer epidermis, which consists of small cells resembling fibers and not typical epidermal cells, and the inner epidermis, which shows signs of collapse. The flared tail is composed of blue staining (unlignified) small cells arranged in a single file.

Staining of sections with phloglucinol-HCl confirmed the results obtained on cell wall lignification of epidermal and fiber cell walls of lemma and palea from toluidine blue staining. Only one micrograph of lemma is illustrated here (Fig. 15) to show the usefulness of this stain. As seen in Fig. 15, fiber tissues and bulk of outer epidermis are stained reddish-pink. The outermost regions of the outer epidermis are stained orangish-pink, and the parenchyma cells remain unstained. The inner epidermis is largely unstained, except in the central region underlying the central vascular bundle, where it abbears reddish-pink.

TEM study was devoted mainly to examining the ultrastructure of outer epidermis, fibers and parenchyma cells (Figs. 16-18). As illustrated in Fig. 16, the outer epidermal wall is extremely thick and appears lamellar. The wall consists of dense and less dense (described here as lucent) lamellae, which are alternating and more or less equally spaced. The dense lamellae are significantly thinner than the lucent lamellae. In some parts of the cell wall lamellae are indistinct, apparently because the section in these parts is oblique and not

transverse relative to the wall. This is not surprising because the outer epidermal walls are extremely thick and highly convoluted, and it is virtually impossible to cut the entire thickness of a wall in a single plane in any one section. Fig. 17 is a TEM view of a cross section through fiber walls. The cell walls are thick and lamellar, consisting of dense and lucent regions. Dense lamellae are very thin. Lucent lamellae are considerably thicker and also more highly variable in their width. As apparent in Fig. 17, lucent lamellae are narrower in inner regions of the cell wall, although the thickness of dense lamellae remains virtually the same throughout the width of the fiber wall. The inter-fiber pits are simple, with long narrow pit canals. TEM view of cross section through parenchyma cells is illustrated in Fig. 18. Parenchyma cells are highly variable in their size and the thickness of their walls. The transverse walls of parenchyma cells are often thinner and traversed by plasmodesmata. The walls in some parenchyma cells are delaminated and/or broken.

Rice husk is a valuable natural resource not only as an excellent source of high quality silica (Sharma et al., 1984; Krishnarao and Godkhindi, 1992; Kalapathy et al., 2002), but also as a source of ligno-cellulosic material which can be potentially used to produce a range of valuable composite products. However, before embarking on products related developments it is important to characterize rice husk in as much detail as possible, and the information provided here should form an useful background in this regard.

A range of microscopy techniques employed provided complementary information on rice husk surface topography, tissue organization and cell wall composition. It is apparent that rice husk (lemma and palea) has unique surface features, with a highly irregular outer surface which is lignified, and a more regular inner surface, which is largely devoid of lignin. The surface features of rice husk described here are similar to those of rice husk surfaces examined earlier (Sharma et al., 1984; Krishnarao and Godkhindi, 1992). The histochemical staining used in our work has provided additional useful information. As the characteristics of the surface of particulate components are known to greatly influence both the properties and performance of composite products (Piggott, 1980), the surface features of rice husk have to be kept in mind when using either the whole husk or its fragments for making composite products. Additionally, tissue composition and stiffness are other important factors that require close consideration.

From the standpoint of topography and composition of cell walls the two surfaces of husk, i.e. the outer epidermis and inner epidermis are likely to differ markedly in their interaction with each other as well as with adhesives used in the manufacture of composite products. Also, these features of two surfaces of husk have to be considered in relation to other factors, such as the temperature and pressure used during the manufacture of composite products based on rice husk.

Although major organic constituents such as cellulose, lignin and hemicellulose of rice husk have been previously determined (Sharma et al., 1984), detailed anatomical and ultrastructural information on internal tissues is lacking. Our study adds a new dimension to understanding tissue organization of rice husk across its thickness as well as its width. In addition, histochemical staining of sections from un-embedded and resin-embedded husks has provided valuable information on cell wall composition of various tissues. TEM observation proved invaluable particularly for cell form and structure of cell walls of parenchyma cells, which were difficult to study by other methods of microscopy, because these cells had extremely thin walls and thus tended to collapse.

From structural perspectives, the husk anatomy is unique. It appears that tissue composition and organization have evolved to provide utmost protection to developing and maturing grains which the husk

encloses. The tight interlocking of lemma with palea is likely to provide an effective seal from atmospheric moisture and microbial invasion. The highly thick-walled and heavily silicified outer epidermal cells together with the underlying layers of fibers undoubtedly provide strength, rigidity and stiffness to husks to combat adverse environmental factors, such as high wind, which may be particularly critical during the stage leading to caryopsis maturation. The unlignified parenchyma and inner epidermal cells no doubt provide flexibility needed for the husk to develop and maintain its desired form.

It is apparent from the microscopic observations presented that rice husk is composed of several types of tissues, which vary greatly in their morphology, structure and composition. From the standpoint of strength, fibers would probably be the most suitable types of cells for use in composite products, but separating them from the rest of the tissues may prove too costly for the products to be cost effective. The use of entire or fragmented husk is therefore an option that is recommended, although for some applications it might be necessary to remove silica from the tissues prior to use. However, it has to be kept in mind that if husk fragments are to be used for composite products, composition and topography of both husk surfaces and their internal tissues are likely to be important factors for the properties and performance of composite products.

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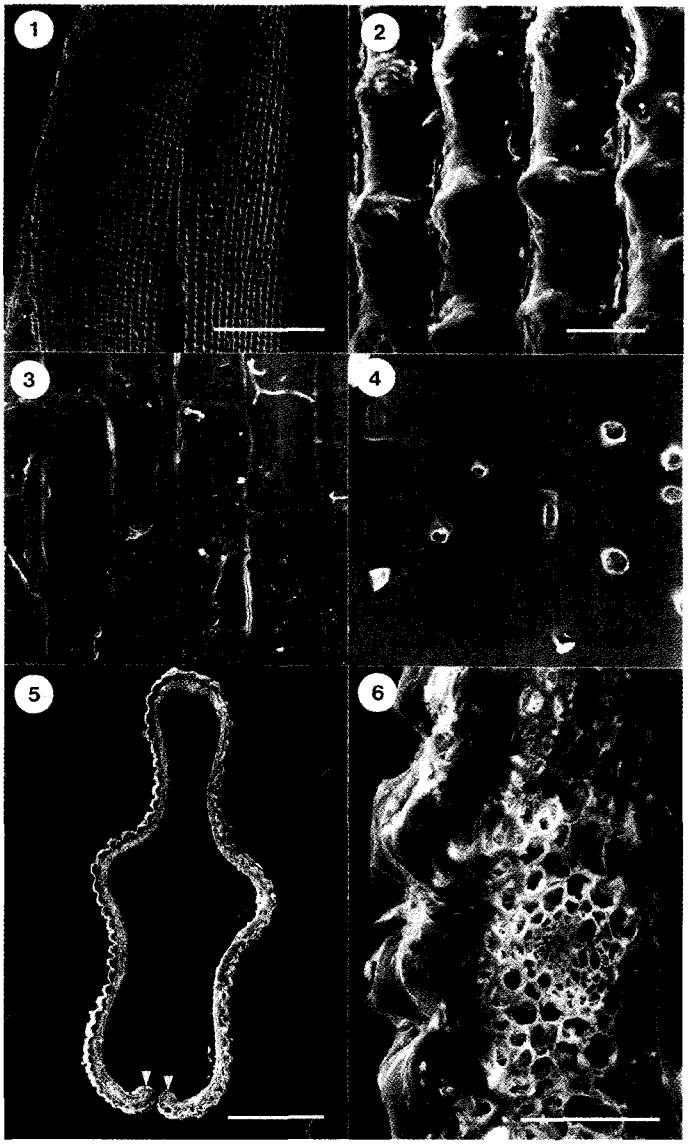


Figs. 1-6. SEM micrographs showing surface features and internal tissue organization of lemma.

Fig. 1. Low magnification view of the outer surface of lemma, which appears highly ridged. The stippled line across the lemma indicates the region used for obtaining sections for light microscopy. Bar = 500 μm.

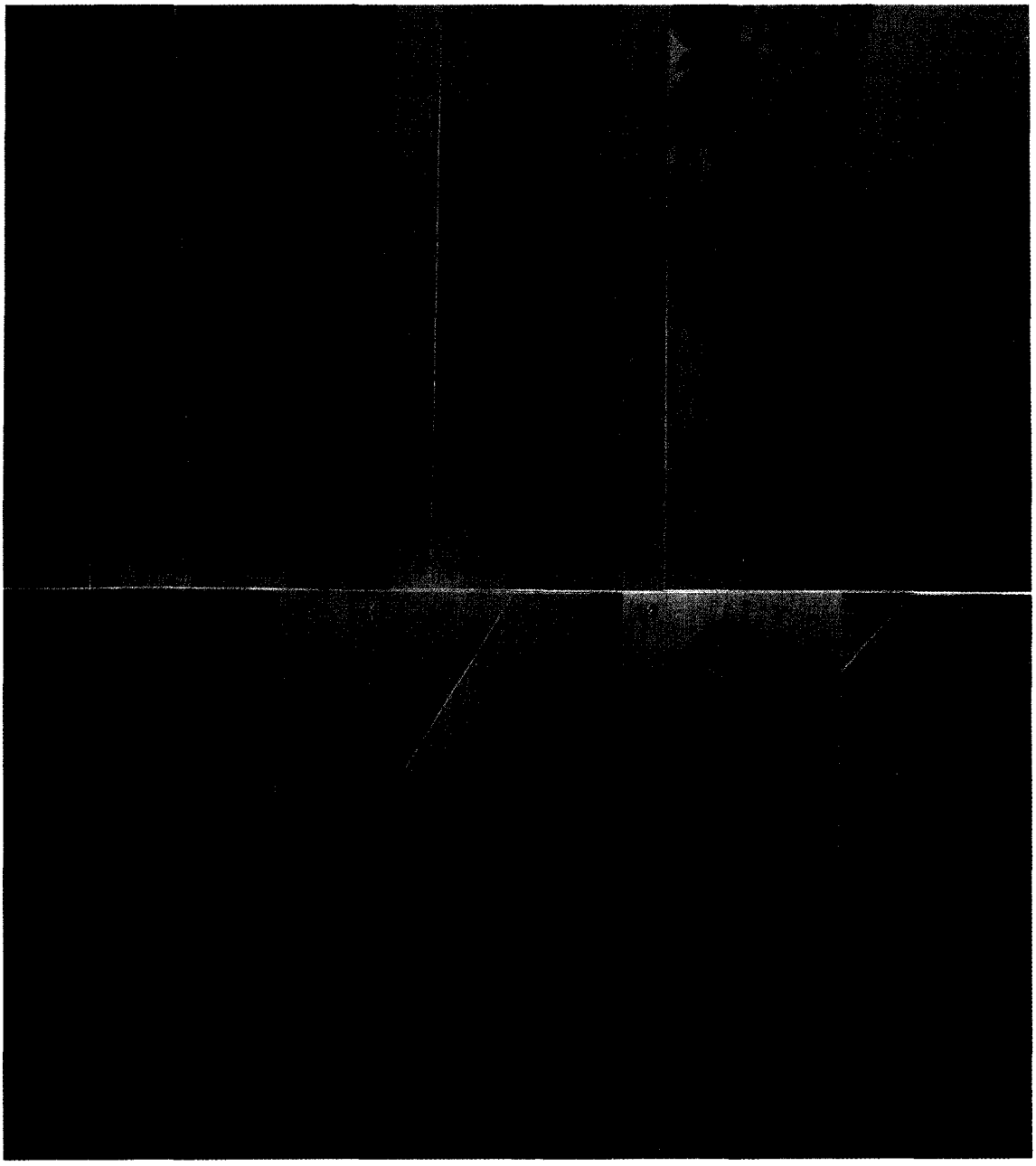
Fig. 2. High magnification view of part of the outer surface of lemma. Epidermal cells are arranged in alternating ridged and unridged linear profiles. Dome-shaped protrusions are a prominent feature of ridges. Bar = 50 μm.

Fig. 3. Inner epidermis of lemma showing a flat profile of elongated cells. Bar = 50 μm.

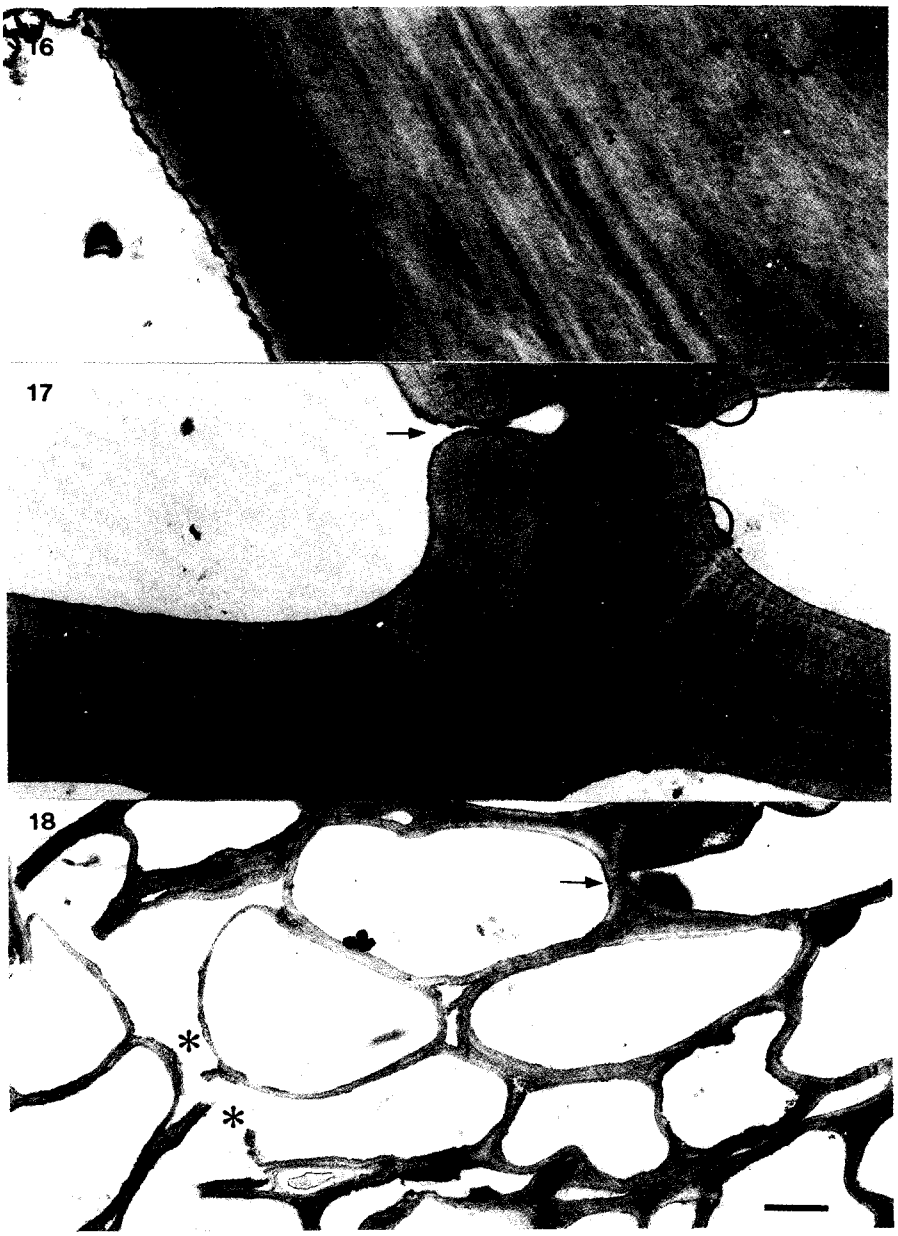
Fig. 4. Inner epidermis of lemma showing stomatal complexes (arrows) and the bases of broken hairs (arrowheads). Bar = 50 μm.

Fig. 5. A cross sectional view of lemma showing undulating outer surface and relatively smooth inner surface. The extremities of margins curl inwards (arrowheads). Bar = 500 μm.

Fig. 6. High magnification view of cross section through central region of lemma showing highly thick-walled outer epidermal cells and a vascular bundle. Xylem (x) and phloem (p) tissues in the vascular bundle are clearly distinguishable. Bar = 50 μm.



Figs.7-15. LM micrographs of cross sections of lemma and palea providing details of internal tissue organization and cell wall composition. All sections were stained with toluidine blue except Fig. 15, which was stained with phloroglucinol-HCl. Fig. 7-11. Cross sections of lemma showing tissue morphology and organization at various magnifications. Fig. 12-14. Cross sections of palea showing tissue morphology and organization at different magnifications. Tissues stained greenish-blue are lignified and those stained bluish are unlignified. Fig. 15. Cross section of lemma stained with phloroglucinol-HCl. Tissues stained orangish-pink are lignified, and those which are not stained are unlignified. Bar = 200 μm for Figs. 7 and 12; Bar = 50 μm for Figs. 8, 9, 13, 14, and 15; Bar = 25 μm for Figs. 10 and 11.



Figs. 16-18. TEM micrographs showing cell wall ultrastructure of various tissues of lemma. Fig. 16. Cross section through the wall of outer epidermal cell. The wall is extremely thick and lamellar, consisting of dense (paired arrowheads) and lucent (arrowhead) lamellae. Lamellar differentiation of the wall is lacking in the region indicated by a star because the section in this part of the wall is oblique. Bar = 1 μm. Fig. 17. Cross section through parts of adjoining fibers. Fiber walls are also thick and lamellar. The dense lamellae (arrowhead) are extremely thin and uniform. In comparison, the thickness of lucent lamellae (star) is highly variable. Lucent lamellae in the circled regions are much thinner than in other regions of the wall. Bar = 1 μm. Fig. 18. Cross section through parenchyma cells. Cell walls are very thin and traversed by plasmodesmata (arrow) in places. The walls are delaminated and broken in some parts (asterisks). Bar = 1 μm.