Microstructure of Cowpea Variety Adua Ayera[1]

S. SEFA-DEDEH and D. W. STANLEY, Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

ABSTRACT

The cowpea (*Vigna unguiculata*), variety Adua Ayera, grown in Ghana, showed all the major anatomical characteristics of legume seeds under the scanning electron microscope. Structures identified include an external cuticle, palisade and mesophyll cells, a double layer of hour-glass cells, and distinct vascular bundles. The seed was characterized by a predominant cotyledon with parenchyma cells containing reserve materials in the form of elliptical starch granules embedded in a protein matrix with protein bodies. Sample preparation was critical. Fixation of the seed in 2% glutaraldehyde prior to sectioning for observation preserved the cotyledon structure with minimal alteration and yielded reproducible results.

Cereal Chem. 56(4):367-371

Legumes play an important role in the diets of a majority of people in the developing countries as both a source of protein and calories. Thus many suggestions have been made to increase research on these seeds aimed at improving their production and utilization.

In studies on the use of cereals and oilseeds for food, the scanning electron microscope (SEM) has been used extensively to understand basic principles involved in their processing (Pomeranz and Sachs 1972, Stanley et al 1976, Sullins and Rooney 1974, Wolf 1970). Unlike cereals and oilseeds, very few SEM studies have been done on the structure of grain legumes (McEwen et al 1974, Rockland and Jones 1974). The objective of this study was to establish, using the SEM, the microstructure of cowpeas (*Vigna unguiculata*), a major legume grown in Africa.

MATERIALS AND METHODS

Cowpeas

The cowpea variety Adua Ayera was obtained from the Crop Science Department, University of Ghana, Legon.

Scanning Electron Microscopy

Unfixed Samples. The cowpeas were mounted on stubs with colloidal silver and coated with an approximately 30 nm layer of gold using a Hummer II sputter coater with gold/palladium (60:40) alloy (Technics Inc., Alexandria, VA).

Fixed Samples. In the search for a suitable method for tissue preparation, two methods of fixation were attempted. The first method involved sectioning cowpeas prior to fixation for 48 hr in glutaraldehyde (2% in 0.025*M* phosphate buffer, pH 6.8) at 4° C. Fixed samples were transferred to the buffer (4° C), which was changed five times over a 1 hr period to remove glutaraldehyde. In the second method, the bean was fixed and washed before sectioning under identical conditions.

The fixed tissues were dehydrated in five 20 min changes of 10, 20, 40, 60, and 80% ethanol and three 20 min changes of 100% ethanol. The dehydrated samples were dried using the critical point drying technique (Anderson 1951), mounted on specimen stubs with colloidal silver, and coated with a 30 nm layer of gold.

The coated samples were viewed and photographed in an ETEC Autoscan scanning electron microscope (ETEC Corp., Hayward, CA) at an accelerating voltage of 20 kV.

RESULTS AND DISCUSSION

Selection of Method for Sample Preparation

It was important that the method selected for preparing samples for observation in the SEM produced no artifacts in the sample. The sample needs to be observed as close to the original state (before sample preparation) as possible, so that the method, once established, could be useful in studying structural changes due to processing, storage, and other physical or chemical treatments.

Cowpea cotyledons sectioned before fixing in glutaraldehyde (Fig. 1) showed a highly disorganized structure. Total destruction of cellular integrity is evidenced from the absence of distinct cell walls and a middle lamella, loosely packed starch granules and protein bodies, and an unstructured protein matrix.

In contrast, cowpea cotyledons sectioned after fixing in glutaraldehyde (Fig. 2) showed a highly organized structure with distinct and compartmentalized cells. A similar structure was reported for faba beans (*Vicia faba*) (McEwen et al 1974) and lima beans (*Phaseolus lunatus*) (Rockland and Jones 1974). In both reports, however, the samples were not fixed and the protein bodies were not clearly identified as in Fig. 2. The technique of fixing the seed in glutaraldehyde before final sectioning for observation is thus recommended. This method preserved the cotyledon structure with minimal alteration and yielded reproducible results.

Seed Anatomy

The cotyledon of cowpeas forms a major part of the seed. It contains parenchyma cells (60 to 100 μm) with reserve materials in the form of elliptical starch granules (11 to 20 μm) (Fig. 3). These

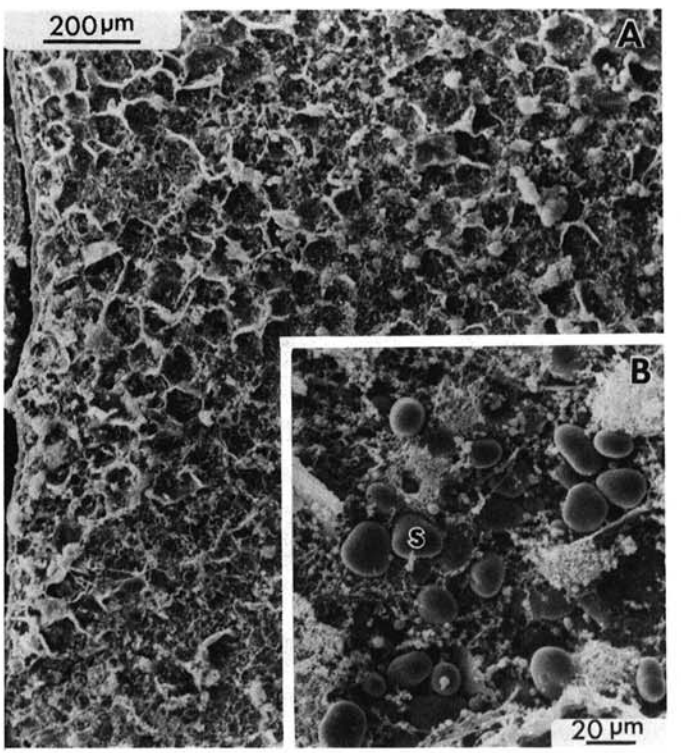

Fig. 1. Cros-section of cowpea, variety Adua Ayera, cotyledon sectioned before fixing in 2% glutaraldehyde. S = Starch granule.

[1]Data are taken from the thesis submitted by S. Sefa-Dedeh to the University of Guelph in partial fulfillment of the requirements for a PhD degree.

0009-0352/79/000058$3.00/0

are embedded in a protein matrix containing protein bodies (3 to 6 μm) (Fig. 4). The protein matrix and the protein bodies were identified in a preliminary study using light microscopy.

The parenchymatous cells of the cowpea cotyledon are bounded by a distinct cell wall and middle lamella (Fig. 5) but, because of the slight swelling that accompanies fixation, these two structures are usually very close together and are difficult to distinguish. Rockland and Jones (1974) could not distinguish boundaries between the cell wall of adjacent cells of raw lima beans. For cowpeas, the structural relation between the cell wall, middle lamella, and intercellular spaces is demonstrated in Fig. 5.

Opik (1966) reported that legume starch grains were very

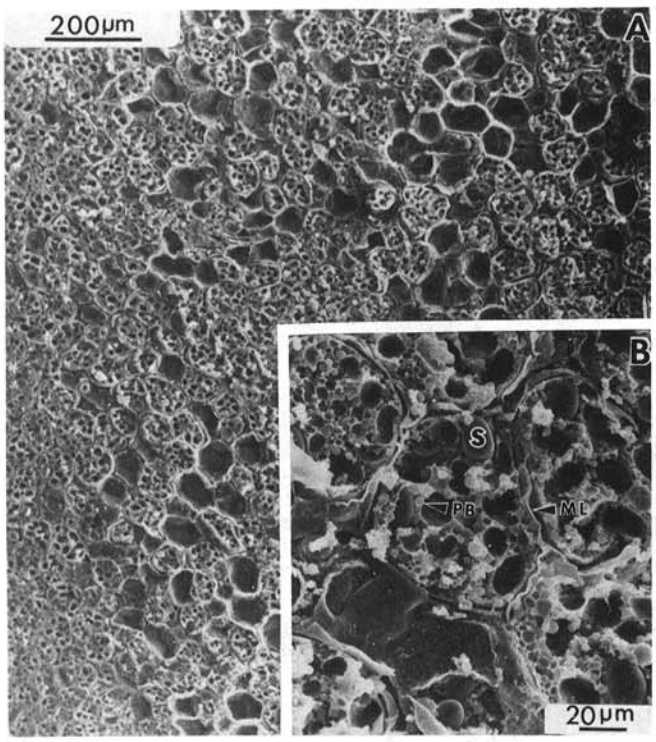

Fig. 2. Cross-section of cowpea, variety Adua Ayera, cotyledon sectioned after fixing in 2% glutaraldehyde. **S** = Starch granule, **ML** = middle lamella, **PB** = protein body.

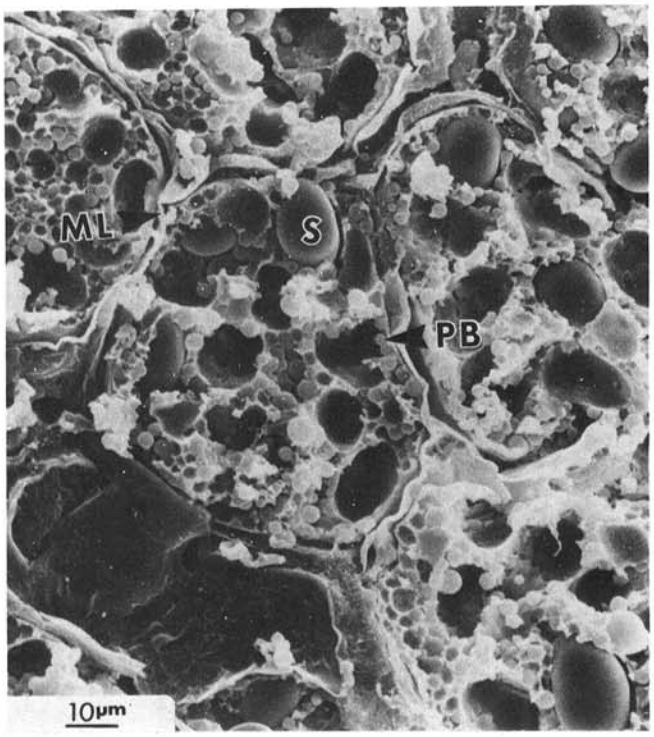

Fig. 3. Cross-section of cowpea, variety Adua Ayera, cotyledon. **ML** = Middle lamella, **PB** = protein body, **S** = starch granule.

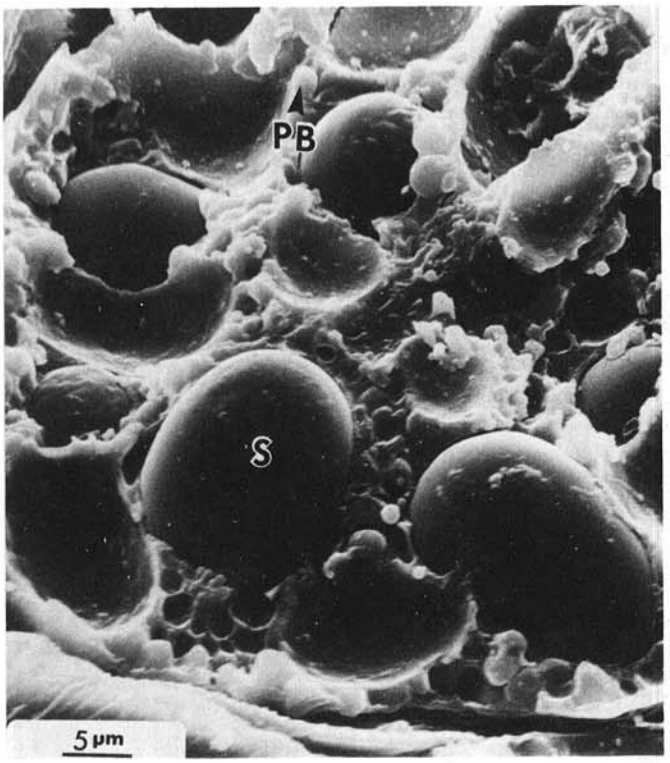

Fig. 4. Cross-section of cowpea, variety Adua Ayera, cotyledon cell. **S** = Starch granule, **PB** = protein body.

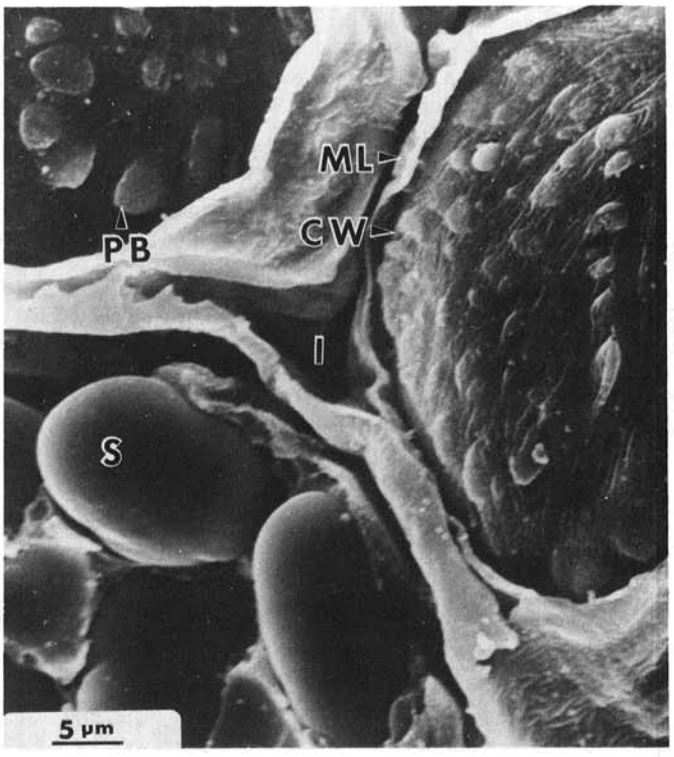

Fig. 5. Cross-section of cowpea, variety Adua Ayera, cotyledon. **I** = Intercellular space, **CW** = cell wall, **PB** = protein body, **S** = starch granule, **ML** = middle lamella.

difficult to section without tearing. The same difficulty was encountered for the cowpea starch grains. To achieve softening of the starch grains, the cowpeas were heated to 100° C prior to fixation. The sections obtained (Fig. 6) showed the internal structure of the starch grains is made up of concentric rings. It is thought that because of the heat treatment, the starch grains lost their spherical shape and become deformed in the process. Hahn et al (1977) reported a similar change in lima bean starch granules and indicated that this granule deformation, initiated in the central portion of the grain, is associated with a loss of birefringence. Hoseney et al (1977), working with wheat starch, also reported a collapsed or folded appearance at the upper limit of gelatinization.

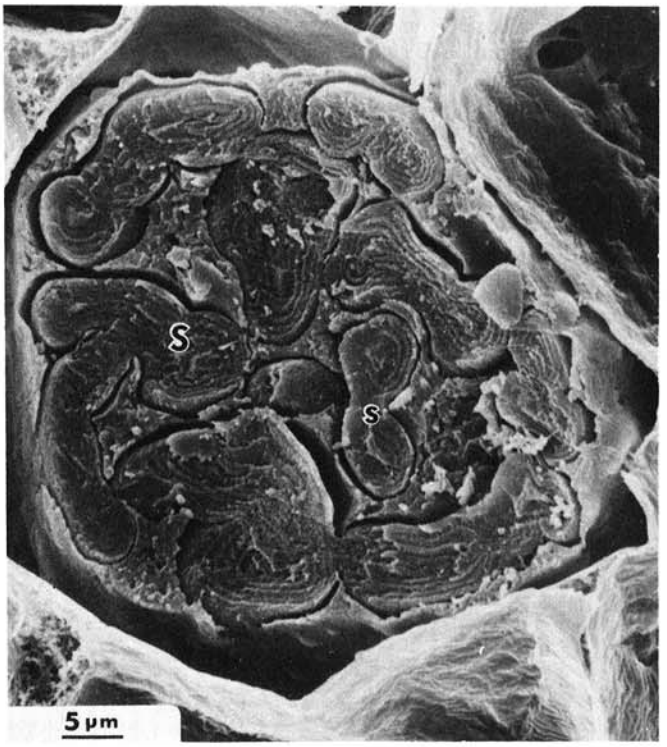

Fig. 6. Cross-section of cowpea, variety Adua Ayera, cotyledon cell showing starch granules with concentric rings. **S** = Starch granule.

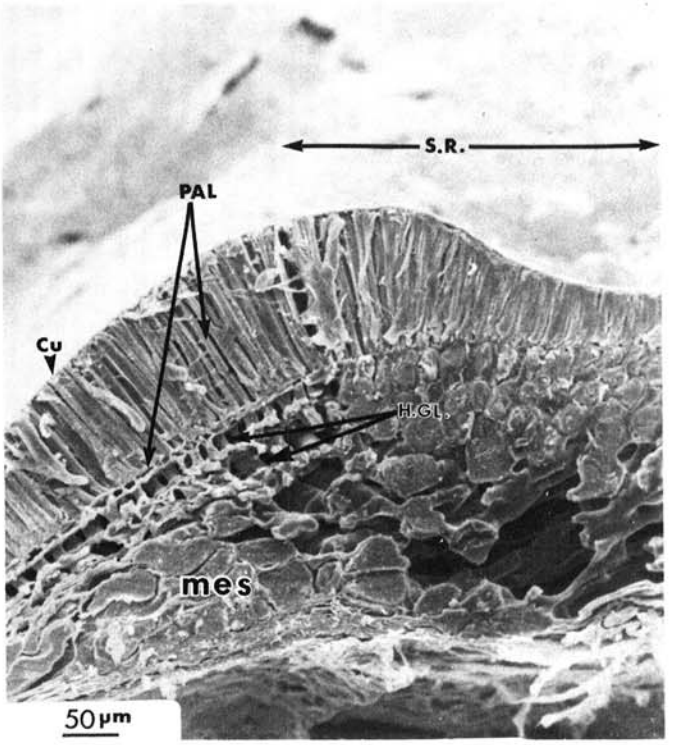

Fig. 8. Cross-section of cowpea seed, variety Adua Ayera, subhilar region. **Cu** = Cuticle, **PAL** = palisade, **SR** = Subhilar region, **HGL** = hour-glass cells, **mes** = mesophyll.

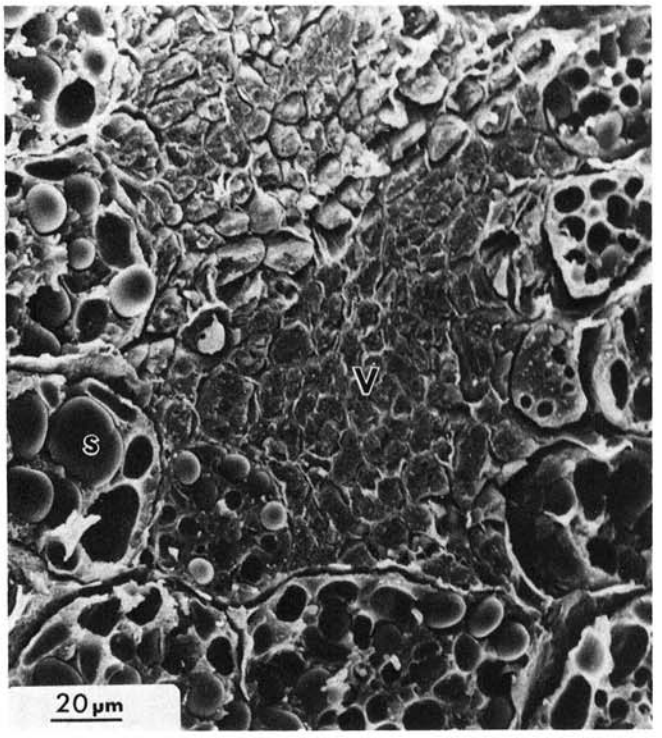

Fig. 7. Cross-section of cowpea, variety Adua Ayera, cotyledon showing vascular bundle. **V** = Vascular bundle, **S** = starch granule.

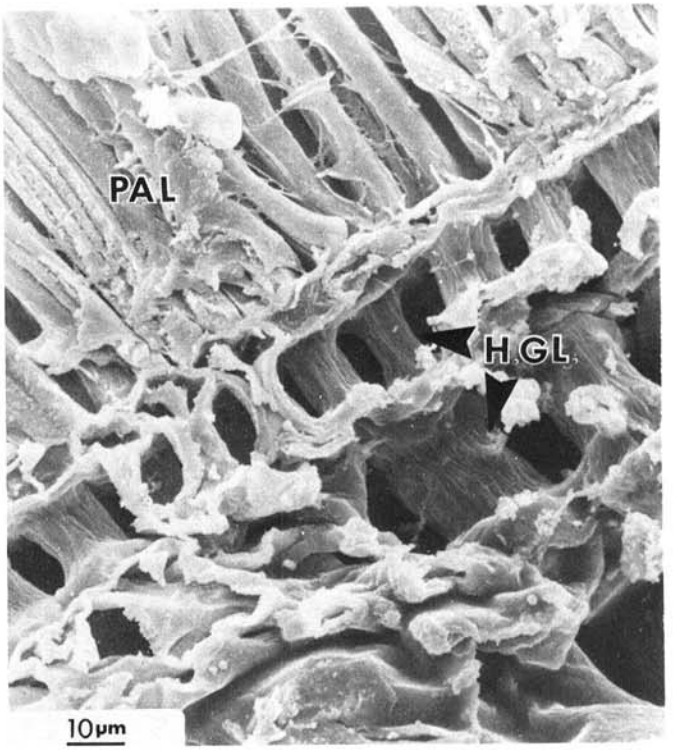

Fig. 9. Cross-section of cowpea seed, variety Adua Ayera, showing hour-glass cells. **HGL** = Hour-glass cell, **PAL** = palisade.

Apart from the parenchymatous cells that fill most of the cotyledon, vascular bundles (Fig. 7) are scattered throughout the cotyledon. The vascular bundle contains a large number of closely packed cells.

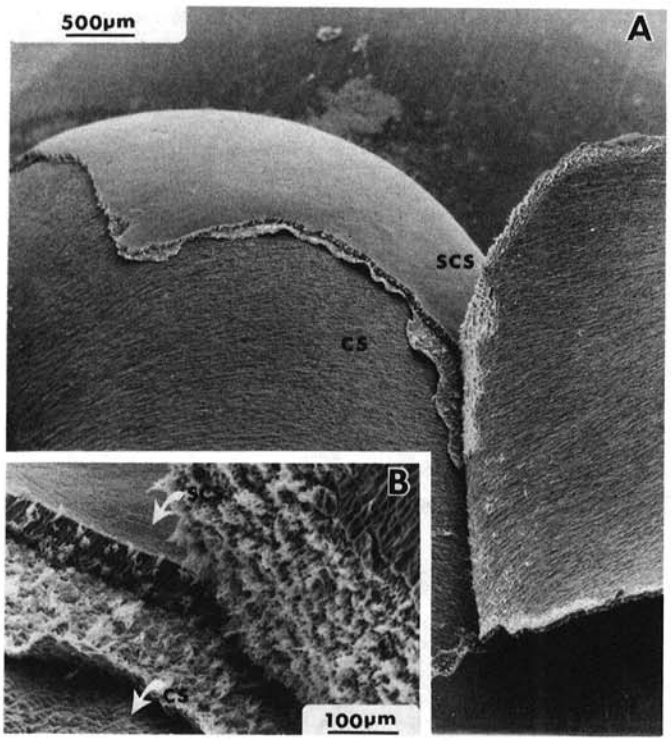

Fig. 10. Cowpea, variety Adua Ayera, seed showing structural relation between seed coat and cotyledon surface (unfixed). **CS** = Cotyledon surface, **SCS** = seed coat surface.

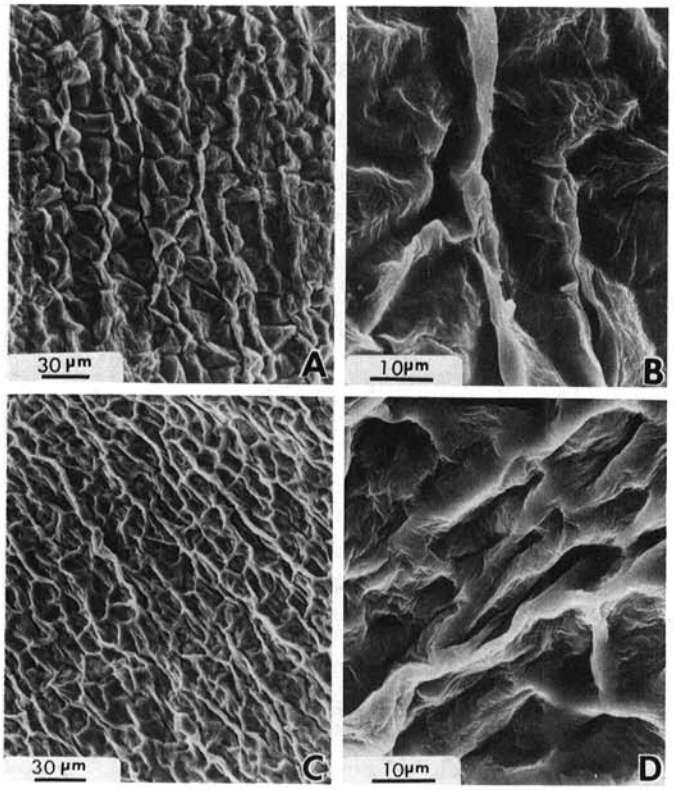

Fig. 11. A and B = Cotyledon surface and **C and D** = inner surface of cowpea, variety Adua Ayera, seed coat.

The cowpea seed testa showed all the major anatomical characteristics of legume seeds as reported by Corner (1951). The outermost layer of the seed coat is the cuticle (Fig. 8). Next to this is the prismatic thick-walled contiguous cells referred to as palisade cells. According to Corner (1951), the hardness and impermeability of dried testa are caused mainly by the contraction of the walls of the palisade cells as the seed ripens. The palisade layer may thus be important in hydration properties.

The hour-glass cells characteristic of legume seeds are situated between the palisade and the stellate mesophyll (Fig. 8). Two distinct layers can be clearly identified (Fig. 9). While both layers are approximately of the same height (25 μm), the thickness of the layer closest to the palisade cells (10 μm) is about half that of the inner layer. Chowdhury and Buth (1970) reported the presence of a single layer of hour-glass cells in most legumes. Only a few species (*Cajanus cajan, Dolichos lablab,* and *D. biflorus*) showed more than one layer of hour-glass cells as we observed in the cowpea.

The seed coat surface of the cowpea variety 'Adua Ayera' is smooth (Fig. 10A), with a thick palisade layer (59.33 ± 1.79 μm). In contrast, the inner structure of the seed coat and cotyledon surface are rough (Fig. 10B). Higher magnification (Fig. 11) revealed details of the surface structure of the cotyledon and the inner surface of the seed coat. The cotyledon surface is covered by wide "hills" with narrow "valleys" (Fig. 11A,B), whereas the inner surface of the seed coat is covered with narrow hills and wide valleys (Fig. 11C,D). This surface topography suggests that the cotyledon surface and the inner surface of the seed coat may be complementary structures, the hills of the former fitting into the valleys of the latter. This interlocking structure of cotyledon and seed coat, which may possibly be strengthened through soaking, is thought to lead to difficulty in dehulling this variety of cowpea by hand rubbing (as practiced in the home) or abrasion.

CONCLUSIONS

The method of sample preparation of cowpea seeds for observation in the SEM is of extreme importance if microstructure studies are to be successful. Fixation of seeds in 2% glutaraldehyde for 48 hr prior to sectioning gave the best results. This method is thus recommended for legume cowpea seed preparation.

The cowpea seed has an organized structure showing all the major anatomical characteristics of legumes. The seed coat structure may play an important role in water absorption and dehulling properties and more work is now required on different cowpea varieties to determine if structural differences are important in processing.

ACKNOWLEDGMENTS

We wish to thank officials of the Ghana-Guelph exchange program for providing the cowpea samples. This research was supported in part by the National Research Council and the Ontario Ministry of Agriculture and Food.

LITERATURE CITED

ANDERSON, T. F. 1951. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. Trans. N.Y. Acad. Sci. Ser. II 13:130.

CHOWDHURY, K. A., and BUTH, G. M. 1970. Seed coat structure and anatomy of Indian pulses. In: ROBSON, N. K. D., CUTLER, D. F., and GREGORY, M. New Research in Plant Anatomy. Academic Press: New York.

CORNER, E. J. H. 1951. The leguminous seed. Phytomorphology 1:117.

HAHN, D. M., JONES, F. T., AKHAVAN, I., and ROCKLAND, L. B. 1977. Light and scanning electron microscope studies on dry beans: Intercellular gelatinization of starch in cotyledons of large lima beans (*Phaseolus lunatus*). J. Food Sci. 42:1208.

HOSENEY, R. C., ATWELL, W. A., and LINEBACK, D. A. 1977. Scanning electron microscopy of starch isolated from baked products. Cereal Foods World 22:26.

McEWEN, T. J., DRONZEK, B. L., and BUSHUK, W. 1974. A scanning electron microscope study of fababean seed. Cereal Chem. 51:750.

OPIK, H. 1966. Changes in cell fine structure in the cotyledons of

Phaseolus vulgaris L. during germination. J. Exp. Bot. 17:427.
POMERANZ, Y., and SACHS, I. B. 1972. Determining the structure of the barley kernel by scanning electron microscopy. Cereal Chem. 49:1.
ROCKLAND, L. B., and JONES, F. T. 1974. Scanning electron microscope studies on dry beans. Effects of cooking on the cellular structures of cotyledons in rehydrated large lima beans. J. Food Sci. 39:342.
STANLEY, D. W., GILL, T. A., DEMAN, J. M., and TUNG, M. A. 1976. Microstructure of rapeseed. Can. Inst. Food. Sci. Technol. J. 9:54.
SULLINS, R. D., and ROONEY, L. W. 1974. The relation between endosperm structure and digestibility of waxy and non-waxy sorghum. Cereal Sci. Today 19:412.
WOLF, W. J. 1970. Scanning electron microscopy of soybean protein bodies. J. Am. Oil. Chem. Soc. 47:107.

[Received October 9, 1978. Accepted February 19, 1979]