A Scanning Electron Microscope Study of Fababean Seed

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ABSTRACT

The cotyledons and seed coat of fababean (*Vicia faba* L.) variety Ackerperle were examined using the scanning electron microscope. Photomicrographs showed no discontinuity in the thick seed coat. Cross-section of the seed coat showed characteristic palisade, parenchyma, tracheid, and hourglass cells, similar to those of other legumes. Examination of the cells in the cotyledons revealed variably shaped starch granules of about 25 to 40 μ in diameter surrounded by irregularly shaped protein bodies of 1 to 5 μ in diameter. The cell walls were about 2 μ thick and had a ribbed or furrowed inner surface. Photomicrographs of the outer surface of the cotyledons and embryo, roller-milled flour, and a protein isolate are also reported.

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Fababean (*Vicia faba* L.) is a potential high protein crop for Western Canada. Although it has been grown in Europe for many centuries, the microscopic structure of the bean has not been completely elucidated. In 1969, Briarty et al. (1) used light and transmission electron microscopy to study the ultrastructure of faba bean cotyledon cells. The scanning electron microscope (SEM), which has been used successfully to examine the microscopic structure of the grain of barley (2), buckwheat (3), oats (4), soybean (5), and wheat (6), has not been applied to fababeans. This article reports results obtained with SEM on the gross and detailed structure of the fababean seed.

MATERIALS AND METHODS

Vicia faba L. (var. Ackerperle) grown on experimental plots at the University of Manitoba in the summer of 1972 was used for this study.

Sample Preparation

Cross-sections of the mature seed coat were prepared by cutting the seed coat with a sharp blade. The dry cotyledons were fractured without rehydration to reveal the contents of the cells.

Preparation of Flour

The beans were milled into flour to pass through a 9XX sieve on an Allis-Chalmers experimental mill.

Starch Preparation

Starch samples were prepared for scanning electron microscopy according to the procedure of Evers (7).

Preparation of Protein Isolate

The flour was dispersed in a dilute (0.016N) NaOH solution, 1:10 (w./v.), pH 8.5, and centrifuged to separate the insoluble residue. The proteins were precipitated from the supernatant solution by adjusting the pH to 4.0 with 1N hydrochloric acid. The precipitate was washed with water and freeze-dried.

Scanning Electron Microscopy

The specimens were mounted on circular stubs with double-sided tape, coated with gold to a thickness of 20 to 25 nm. and examined on a Cambridge "Stereoscan" MK IIA SEM at an accelerating potential of 10 kv. Selected areas were photographed on Panatomic X film.

RESULTS AND DISCUSSION

The seed coat of fababeans, which constitutes about 15% of the weight of the bean, provides considerable protection for the enclosed cotyledons and embryo. The smooth outer surface of the seed coat is shown in Fig. 1a. Also shown in this figure is the hilum or seed scar, the point of attachment of the funiculus to the seed. This region measures about 1 to 5 mm. and is slightly elevated from the surface of the seed coat. The hilum is characterized by the black color of the surface tissue and the central groove running through its entire length. This

groove opens or closes depending on the relative amounts of moisture inside and outside the bean, thus playing an active role in the dehydration process of the bean. The hilum is located along the mid-line of the bean and lies just beyond the one end of the axial tip of the embryonic root or radicle.

Figure 1b shows in more detail one end of the hilum and surrounding seed coat. The material shown at the end of the hilum is probably a portion of the funiculus which remained attached to the bean. Figure 1c is a higher magnification of the tissue on the surface of the hilum. This material appears flaky and folded with no regular pattern.

At high magnification, the outline of the surface cells of the seed coat can be seen (Fig. 1d). Examination of large areas of the seed coat indicated neither surface damage nor any of the prominent funnel-shaped pits seen in soybean seed coats (5).

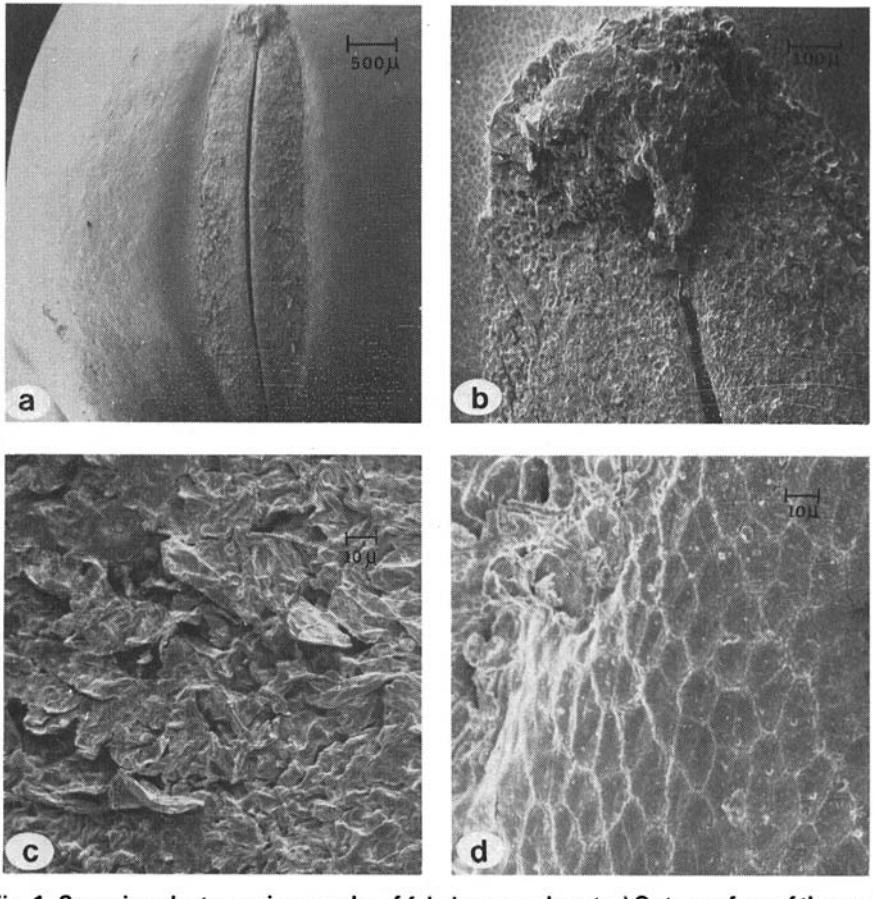


Fig. 1. Scanning electron micrographs of fababean seed coat. a) Outer surface of the seed coat and the hilum; b) and c) enlarged view of the hilum surface; d) enlarged view of the seed coat surface.

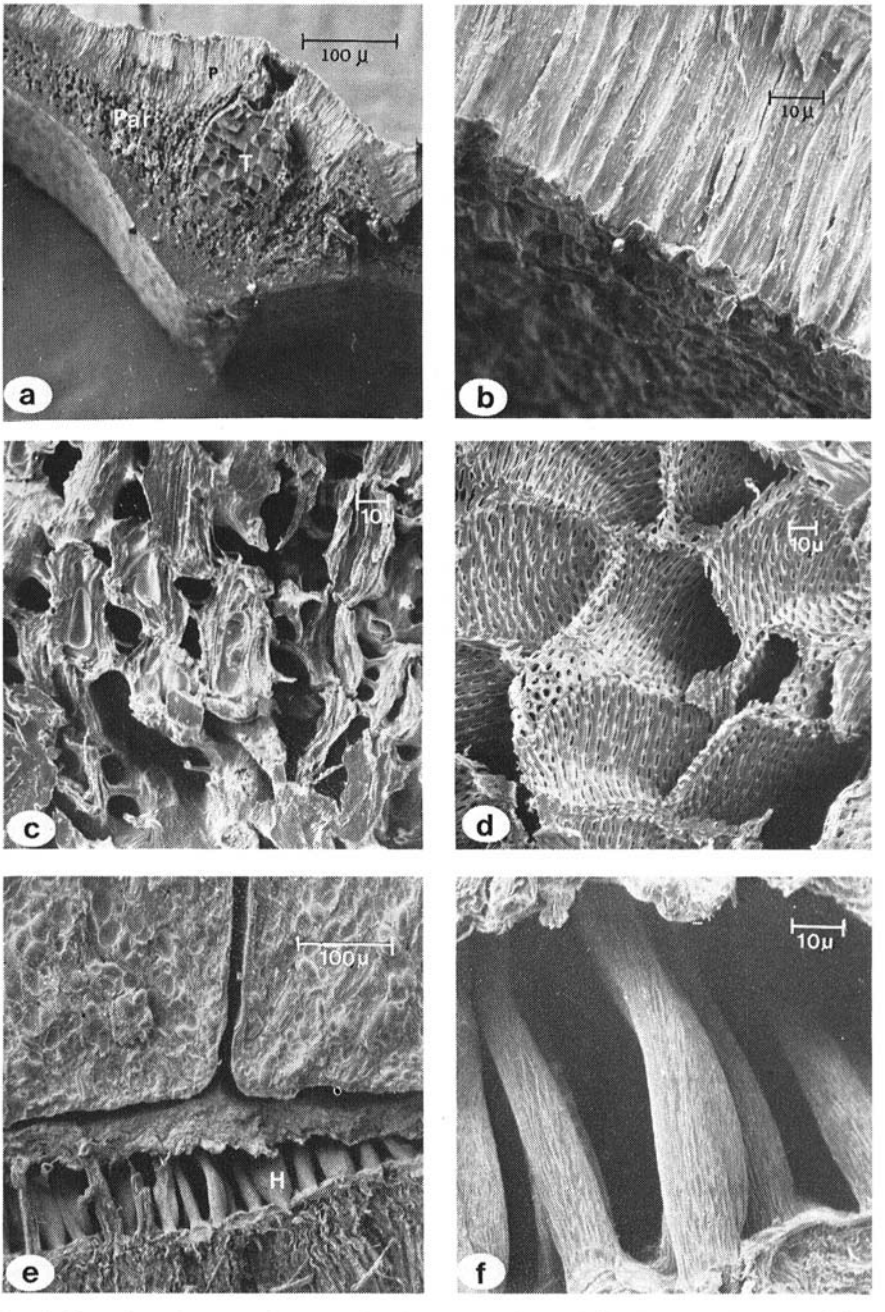


Fig. 2. Scanning electron micrographs of cross-sections of fababean seed coat: a) Hilum region showing palisade cells (P), parenchyma cells (Par), and tracheid cells (T); b) enlarged view of palisade cells; c) enlarged view of parenchyma cells; d) enlarged view of tracheid cells; e-f) hourglass cells (H) in the seed coat away from the hilum.

A cross-section of the seed coat in the hilum region showed several distinct types of tissue (Fig. 2a). Immediately under the flaky surface layer of the hilum is a double layer of palisade cells, each measuring about 30 to 40 μ in height. The outer layer of these cells extends only to the edge of the hilum whereas the inner layer appears to be continuous with the single palisade layer found throughout the seed coat. Below the central groove of the hilum are the tracheid cells through which nutrients enter the maturing bean. Around the tracheids are loosely and irregularly structured parenchyma cells. Closer to the inner surface of the seed coat, these parenchyma cells become highly compacted.

A magnified view of the palisade cells (Fig. 2b) shows their fibrous nature. The highly irregular and loose arrangement of the parenchyma cells can be seen in Fig. 2c. The mesh-like arrangement of the tracheid cells can be seen in detail in

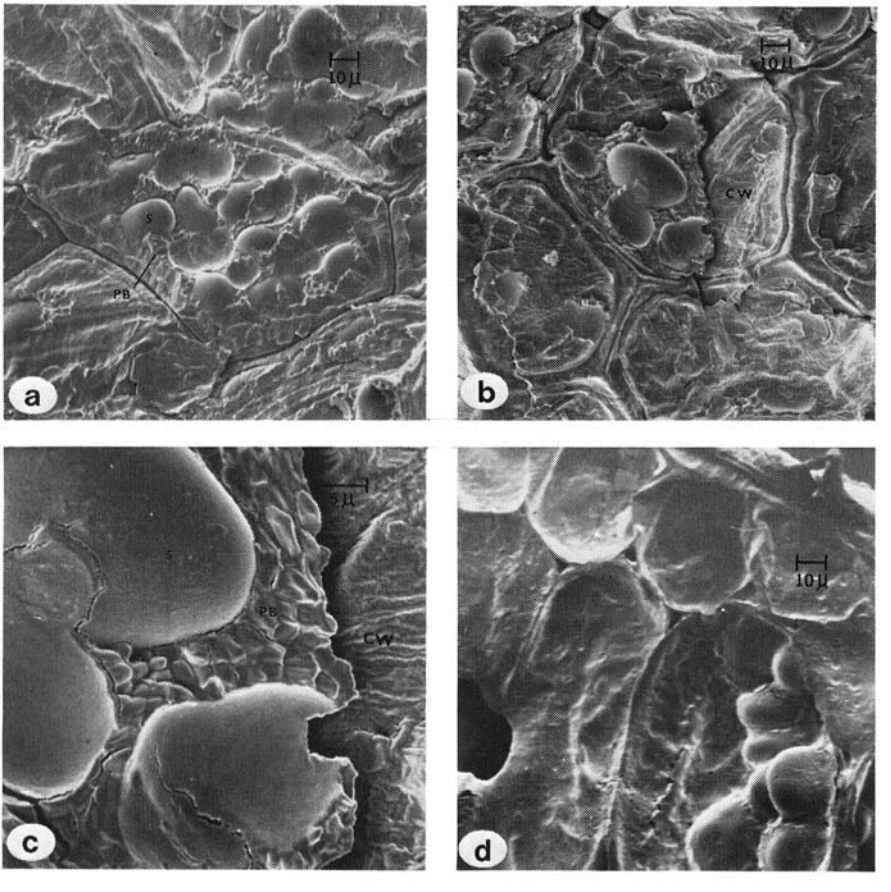
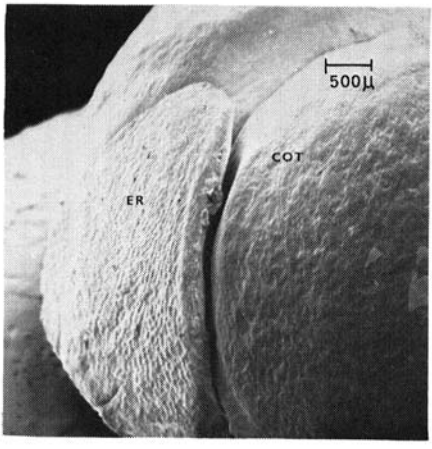


Fig. 3. Scanning electron micrographs of fababean cotyledon cells: a-b) Structure showing starch (S), protein bodies (PB) and cell walls (CW); c) enlarged view of the protein bodies and the interior surface of the cell wall; d) surface cell layers after leaching with water.



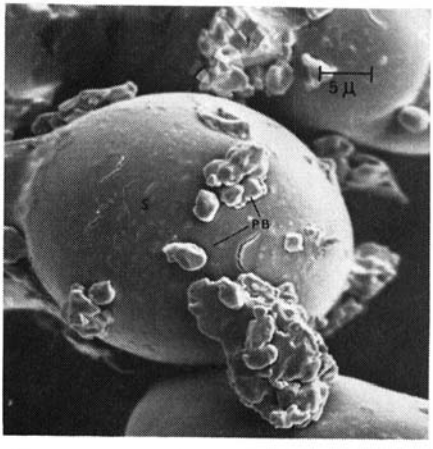


Fig. 4 (left). Scanning electron micrograph of a fababean seed with the seed coat removed showing the cotyledons (COT) and the embryonic root (ER).

Fig. 5 (right). Scanning electron micrograph of fababean flour.

Fig. 2d. These cells are very similar to those observed in soybean (5).

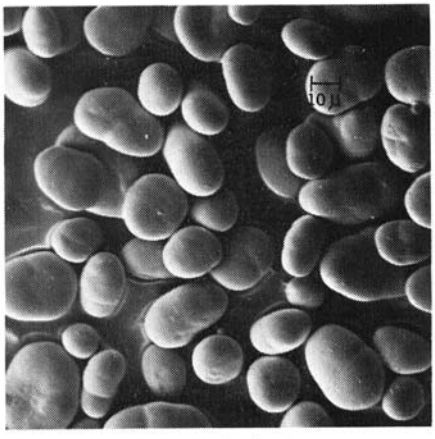
In regions away from the hilum, between the palisade and parenchyma cells, is another type of cell, generally referred to as the hourglass cells (Fig. 2, e and f). These cells show a variety of shapes and sizes, ranging in length from 10 to almost 100 μ. A close examination of these cells revealed parallel ridges along the longitudinal axis (Fig. 2f). Figure 2e also shows portions of the two cotyledons in which outlines of the storage cells and embedded starch granules can be seen.

Additional features of the internal structure of cotyledon cells are shown in Fig. 3a and b. These cells are angular in shape and measure approximately 100 to 150 μ in diameter with cell walls about 2 μ thick. The somewhat irregularly shaped protein bodies appear to range in size from 1 to 5 μ in diameter (Fig. 3c). They are similar in size, but not in shape, to the smaller of the two types of protein bodies reported for soybean (5). Also shown in Fig. 3c is a higher magnification of the inner surface of the cell wall. The most striking feature seen here is a ribbed or furrowed surface.

Figure 3d shows the marked difference in size of the surface layer of cotyledon cells and interior cells. This specimen was cut from the cotyledon after soaking in water for 3 hr.; most of the cell contents of the surface cells were lost during cutting. A few starch granules with some adhering material (probably protein) can be seen in the large cell in the lower right-hand corner of the photograph. Repeated water washing of the cut surface completely removed the cell contents revealing the furrowed inner surface of the cell wall.

The most prominent constituents of intact cotyledon cells are the starch and protein which constitute about 47 and 34% of the cotyledon by weight, respectively[2]. Although it is somewhat difficult to determine with the SEM, both light[2] and transmission electron (1)[2] photomicrographs indicate that most of the

[2]Unpublished data.



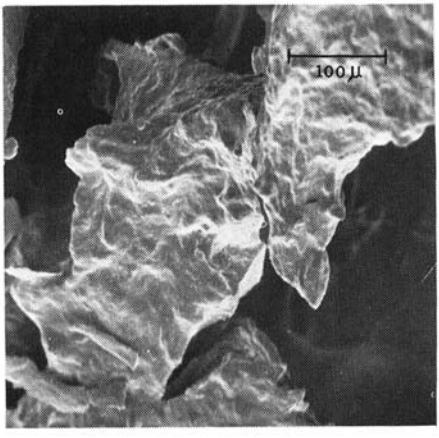


Fig. 6 (left). Scanning electron micrograph of fababean starch.

Fig. 7 (right). Scanning electron micrograph of fababean protein isolate.

protein is localized in discrete protein bodies. This is in accord with chemical studies which indicate vicilin and legumin, the two storage proteins, make up about 80% of the protein (8), and that these two globulins are found exclusively in the protein bodies (9).

At low magnification, the outer surface of the cotyledons appears relatively smooth with no noticeable pitting or surface cracks (Fig. 4). Also seen in this photomicrograph is the embryonic root in the cleft between the two cotyledons.

Photomicrographs of the bean flour indicated that the starch granules remained intact upon milling, whereas the protein was found to range from individual protein bodies to aggregates of 20 to 30 μ containing many protein bodies (Fig. 5). Although a few protein bodies and cell wall fragments could be found adhering to the starch granules, there did not appear to be a matrix protein of the type reported for wheat (6) or barley (2).

Figure 6 shows a starch sample prepared by repeated water washing of the flour. The granules ranged in size from 10 to 40 μ in length and from 10 to 25 μ in width. Most of the smaller granules were slightly oblong, while the larger ones appeared to be made up of two or three lobes. In this regard, fababean starch is quite similar to other bean starches (10).

Preparation of protein isolates by alkali extraction followed by acid precipitation at the isoelectric point resulted in completely amorphous particles exhibiting no evidence of the original structure of the protein bodies (Fig. 7). None of the spherical shapes reported by Wolf and Baker (11) for spray-dried soybean protein isolates were observed. There was no indication of the fibrous strands of protein reported by Orth et al. (12) for freeze-dried glutenin fractions isolated from wheat flour.

Although the detailed structure of the contents of the cotyledon cells cannot be shown with the SEM, this study does present new information on the macrostructure of these cells. Additional studies by the authors are presently being conducted using transmission electron microscopy to further elucidate the

microstructure, and to examine the mechanism of protein accumulation and deposition in the faba bean cotyledon.

Acknowledgments

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Literature Cited

1. BRIARTY, L. G., COULT, D. A., and BOULTER, D. Protein bodies of developing seeds of *Vicia faba*. J. Exp. Bot. 20: 358 (1969).
2. POMERANZ, Y., and SACHS, I. B. Determining the structure of the barley kernel by scanning electron microscopy. Cereal Chem. 49: 1 (1972).
3. POMERANZ, Y., and SACHS, I. B. Scanning electron microscopy of the buckwheat kernel. Cereal Chem. 49: 23 (1972).
4. POMERANZ, Y., and SACHS, I. B. Scanning electron microscopy of the oat kernel. Cereal Chem. 49: 20 (1972).
5. WOLF, W. J., and BAKER, F. L. Scanning electron microscopy of soybeans. Cereal Sci. Today 17: 124 (1972).
6. ARANYI, C., and HAWRYLEWICZ, E. J. Application of scanning electron microscopy to cereal specimens. Cereal Sci. Today 14: 230 (1969).
7. EVERS, A. D. Scanning electron microscopy of wheat starch. III. Granule development in the endosperm. Staerke 23: 157 (1971).
8. BOULTER, D., and DAVIS, O. J. Nitrogen metabolism in developing seeds of *Vicia faba*. New Phytol. 67: 935 (1968).
9. GRAHAM, T. A., and GUNNING, B. E. S. Localization of legumin and vicilin in bean cotyledon cells using fluorescent antibodies. Nature 228: 81 (1970).
10. HALL, D. M., and SAYER, J. G. A scanning electron microscope study of starches. III. Miscellaneous starches. Textile Res. J. 41: 880 (1971).
11. WOLF, W. J., and BAKER, F. L. Scanning electron microscopy of soybean protein products. Cereal Sci. Today 18: 292 (1973).
12. ORTH, R. A., DRONZEK, B. L., and BUSHUK, W. Studies of glutenin. IV. Microscope structure and its relations to breadmaking quality. Cereal Chem. 50: 688 (1973).

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