EXPLORING BUFFALO GOURD SEEDS WITH SCANNING ELECTRON MICROSCOPY[1]

M. TU,[2] C. W. DEYOE,[2] and W. D. EUSTACE,[2] Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506

ABSTRACT

Cereal Chem. 55(5): 773-778

Scanning electron microscopy was used to study seed structure of a xerophilic buffalo gourd (*Cucurbita foetidissima*). By use of solvent extraction and trypsin treatment, protein bodies and oil-containing spherosomes were found to be two major organelles in the seed cotyledons.

Buffalo gourd (*Cucurbita foetidissima* HBK) has been investigated as a valuable source of oilseed protein (1–4). The Ford Foundation has studied it extensively as an economically and nutritionally valuable crop suitable for arid lands in the Middle East and other dry lands in the world (5).

Information on buffalo gourd seed structure is sparse. Hensarling et al (6) used small areas of a specimen and transmission electron microscope to investigate the storage structure of the seed. We chose scanning electron microscopy (SEM) to examine the gourd seed structure. The availability of SEM makes possible the study of three-dimensional structures with practical magnifications ranging from ×20 to ×20,000. The viewed area of the specimen can be much larger than with transmission electron microscopy, and preparing specimens is simple (7,8).

SAMPLE PREPARATION AND EXAMINATION

The buffalo gourd seeds (protein 33%, fat 28%) were from Lebanon. Seed structures were studied and compared with SEM after the following treatments: 1) none, 2) petroleum ether extraction for 8 hr, and 3) petroleum ether extraction for 8 hr followed by 10% trypsin enzyme solution digestion for 8 hr.

The seed was sliced with a razor blade, treated, and attached to a circular (9 mm diameter) specimen stub with plastic cement. The mounted specimen was coated with a 100–200-Å layer of gold by a high-vacuum electron-evaporation apparatus (Kinney Corporation, Boston, MA). The sample was examined by an autoscan SEM (ETEC Corporation, Hayward, CA) at an accelerating potential of 20 kV and photographed on Polaroid film.

RESULTS AND DISCUSSION

The bottle-shaped seed was a pale cream color and about 0.8 cm long. The seed coat was extremely rigid and contained two prominent cotyledons (Fig. 1A and B).

[1]Contribution No. 961-j, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506.

[2]Graduate research assistant, research scientist, and associate professor, respectively, Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506.

0009-0352/78/ 000088$03.00/0

Seed Coat

The outer epidermis consisted of epidermal ribs, palisade cells, and pitted parenchyma cells (Fig. 1C). The epidermal ribs were attached on the epidermis wall and branched toward the outside.

Seed Cotyledons

A perisperm layer consisting of a few layers of small cells was next to the seed coat (Fig. 1D). Under the perisperm layer was an endosperm cell layer. The internal cotyledon cells were mainly regularly arranged parenchyma cells (Fig. 1D and E). Inner parenchyma cells were filled with rough intracellular particles 1–7 μm in diameter (Fig. 1D–G). Those particles were embedded by the spherosome matrix (8,9), which contained the reserve oil of oilseeds. Higher

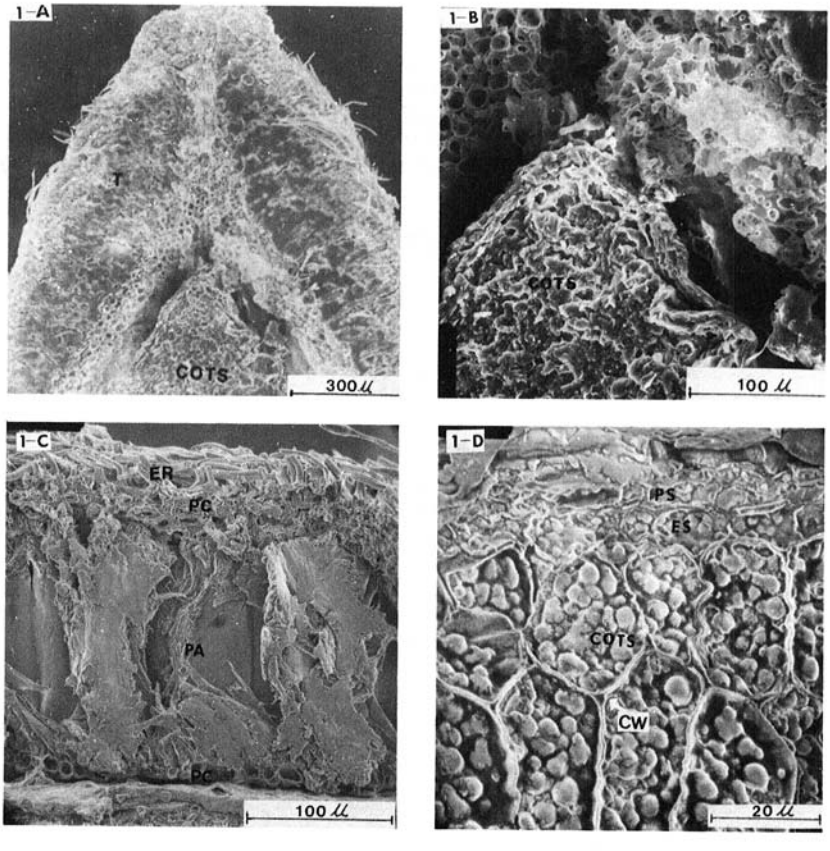


Fig. 1A–D. Longisection of buffalo gourd seed showing testa (A) and cotyledon (B) areas, cross section of seed coat (C), high magnification of cotyledon structure (D). Arrow in D shows juncture of three cotyledon parenchyma cells. T, testa; COTS, cotyledons; ER, epidermal ribs; PA, palisade cells; PC, parenchyma cells; PS, perisperm; ES, endosperm; CW, cell wall of cotyledon cells.

magnification (Fig. 1G) shows that the spherosomes and possibly the cytoplasmic network cover the surfaces of the intracellular particles (8). The abundant intracellular particles were identified as protein bodies by morphologic evidence after extraction of lipids with petroleum ether plus trypsin enzyme digestion. As shown in Fig. 2A–C, no spherosomes surround the intracellular particles after petroleum ether extraction. Moreover, their surfaces were attacked and damaged by trypsin enzyme—proof that the abundant cell bodies are protein bodies of seed cotyledons.

Defatted Seed Cotyledons

The spherosomes containing reserve oil apparently were removed efficiently by petroleum ether extraction (Fig. 3A). Higher magnfication of the cotyledon protein bodies show a smoother naked surface (Fig. 3B and C) than that of the original seed structure (Fig. 1G).

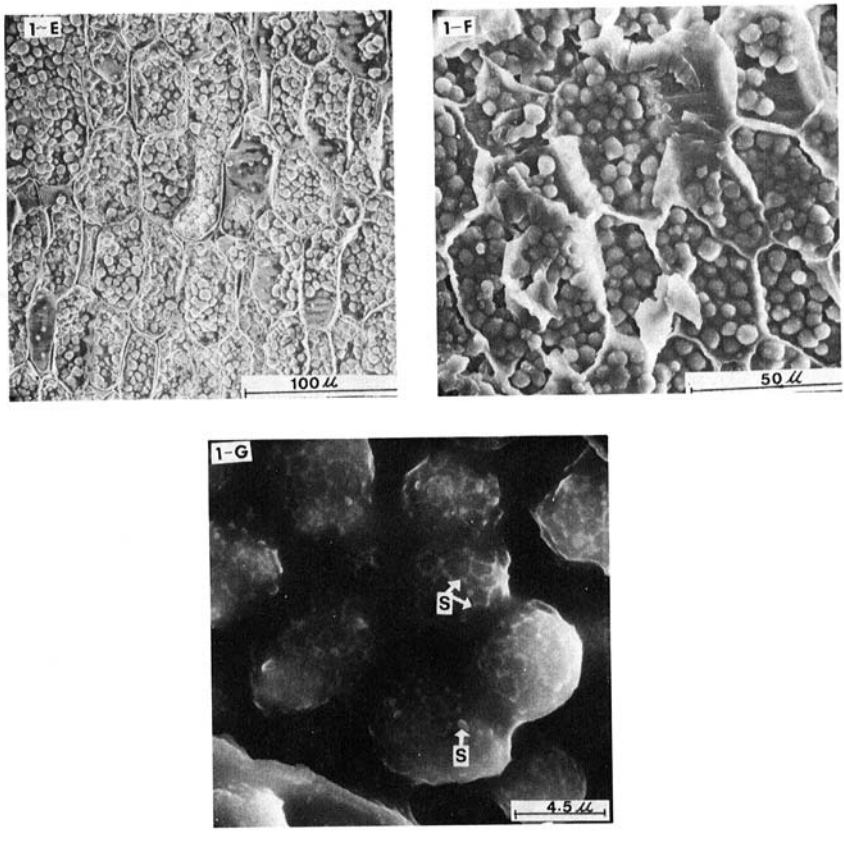
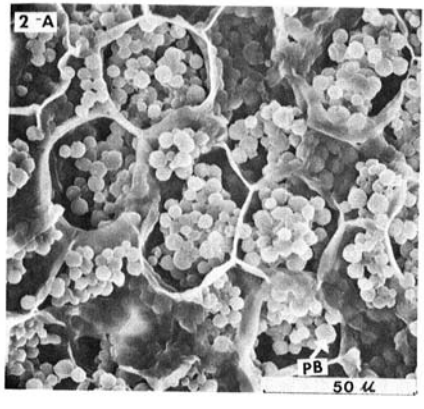
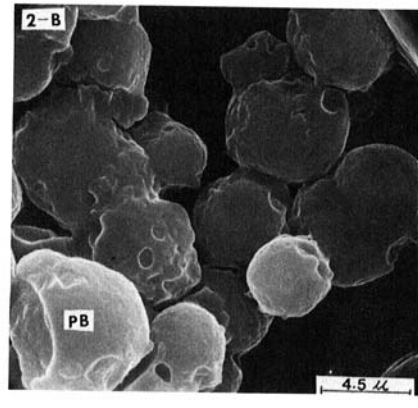


Fig. 1E–G. Longisection of buffalo gourd seed showing high magnification of cotyledon structure. S, spherosomes.

SUMMARY

SEM is a useful tool in studying the seed structure of buffalo gourd. A longisection of the seed coat was observed, and the epidermal ribs, palisade cells, and pitted parenchyma cells were identified. The structure within seed cotyledons also was studied. Protein bodies and oil-containing spherosomes apparently are two major organelles in the seed cotyledons. Oil-containing spherosomes were removed easily by petroleum extraction, and protein bodies were then identified by trypsin attack. The abundance of protein bodies and spherosomes of seed cotyledons indicates that buffalo gourd seeds are protein-rich oilseeds. Results of this investigation agree with a previous report (6) that buffalo gourd seed cotyledon structure is similar to that of other such oilseeds as soybean, peanut, and cotton.





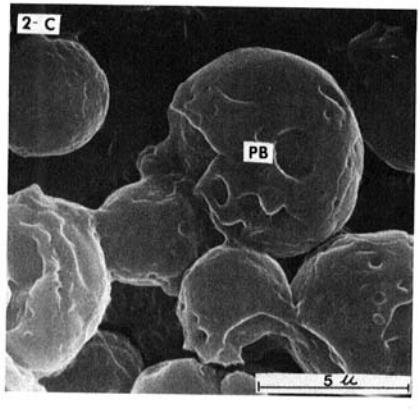
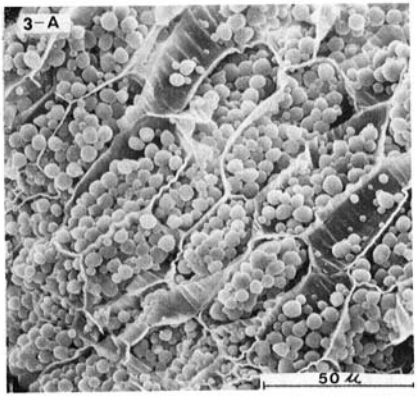
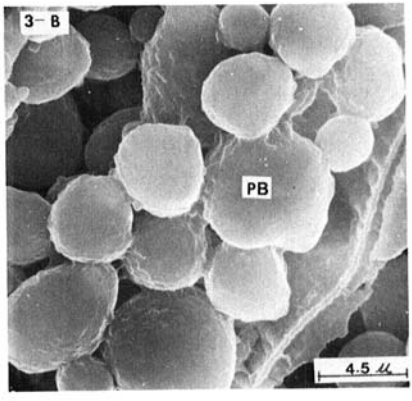


Fig. 2A–C. Protein bodies (PB) in cotyledon cells after petroleum ether extraction for 8 hr and 10% trypsin enzyme solution digestion for 8 hr. Trypsin damaged cell bodies' surface.





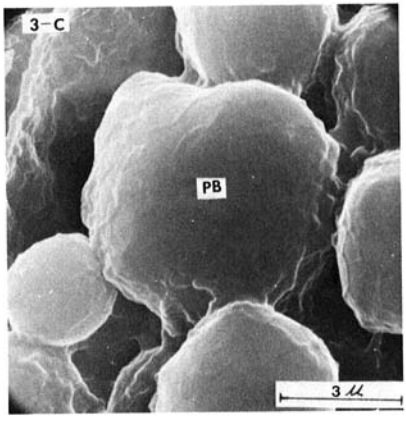


Fig. 3A–C. Cotyledon cells after petroleum ether extraction for 8 hr. Spherosomes containing reserve oil were removed by solvent to show smooth surface structure of protein bodies (PB).

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[Received March 17, 1977. Accepted February 9, 1978]