# Technical News Feature

## Cell-Wall Architecture of Peanut (*Arachis hypogaea* L.) Cotyledon Parenchyma Cells and Resistance to Crushing

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### ABSTRACT

Light microscopy of peanut cotyledon tissue showed some unusual profiles that were interpreted to be cell walls. Comparable tissue was treated with enzymes to eliminate cellular contents and examined in a scanning electron microscope. These parenchyma cells had walls which were bizarre, resembling scalariform xylem elements. Crushing tests on tissue whose cellular contents were removed showed that these cells provided insignificant resistance to crushing.

### INTRODUCTION

A method to produce "partially defatted peanuts" was devised recently at this laboratory (1). Peanuts, which contain 30-35% oil, were squeezed in a hydraulic press to remove most of the oil. The mashed peanuts were then treated with hot water or steam to restore their original form. These reconstituted peanuts were then roasted to give a product with fewer calories but which were just as (or even more, according to some) delicious as the original. The product is now commercially available.

During commercial production, it was found that an occasional lot of peanuts required an unusual amount of pressure to express the oil. Because these difficult-to-press (DP) lots of peanuts are a source of wasted energy and effort, it was deemed desirable to find a way to predict which lots would be difficult to press before they reached the mill. One of the investigations initiated to study this problem was a microscopic examination of peanuts to see if any differences existed between normal and DP peanuts.

Examination of peanuts in the light microscope revealed an unusual staining pattern in certain places within the seed. These places were interpreted to be glancing sections of cell walls. In order to get a clearer idea of the cell wall morphology, peanut coytledon tissue was treated with enzymes to digest away the cellular contents so that the wall itself could be visualized in the scanning electron microscope (SEM). This paper presents the results of our findings, which show that the peanut cotyledon cell walls have a strange architecture and that the cell wall material itself could not account for the extra pressure required by these DP peanuts.

### MATERIALS AND METHODS

#### Light Microscopy

Pieces of tissue about 2 $mm^3$ were cut from peanut cotyledons and fixed in 4% glutaraldehyde prepared in 0.05 M cacodylate, pH 7. They were left in the fixative over the weekend at room temperature. The tissue was rinsed and post-fixed in 1% $O_sO_4$/0.05 M cacodylate, pH 7. The tissue was rinsed in water and dehydrated in a graded series of aqueous ethanol, transferred to acetone and infiltrated with a graded series of Spurr's epoxy resin in acetone. The resin was polymerized in a 60 C oven overnight and sections about 1 to 5 $\mu$m thick were cut from the block. The sections were placed onto a glass slide and stained with toluidine blue (2) and viewed in a light microscope.

#### Scanning Electron Microscopy

Small sections of cotyledon tissue were cut by hand with a razor blade and treated with an enzyme solution over the weekend at 35 C. the enzyme solution consisted of 30 mg "pronase" (Calbiochem) per 10 ml 0.05 M tris buffer, pH 8.3, that contained 10% ethanol with 0.01 M calcium ions (3). After the enzyme treatment, the sections were rinsed several times in distilled water and then lyophilized. The freeze-dried tissue was coated with gold in a Hummer II Sputtering System (Technics, Alexandria, VA) to a thickness of about 1 to 1.5 Å and the cut edge was viewed in an SEM Super II (ISI, Mountain View, CA).

#### Crushing Test

Small blocks, about 3 $mm^3$, were cut from cotyledon tissue with a razor blade and defatted in hexane that contained 2-3% acidified 2,2-dimethoxypropane for about 20 hr (4). The defatted pieces were then placed into a collodion bag together with enzyme solution as above; the bag was suspended in 0.05 M tris, pH 8.3, that contained 0.01 M calcium ions and 10% ethanol, for 5 days at room temperature. The pieces of tissue were then rinsed in distilled water and lyophilized. The lyophilized pieces were crushed in an Instron compression cell on the Instron instrument.

### RESULTS AND DISCUSSION

Figure 1 is a light micrograph of a transverse section through a peanut cotyledon. The arrow in Figure 1 points to a peculiar structure that we interpreted to be a tangential view of a cell wall with unusual sculpturing. This type of architecture was observed in certain sections that were cut right at the edge of a cell. This unusual type of cell-wall structure seemed out of place in the midst of storage parenchyma cells. We have seen xylem vessels with scalariform walls in cotyledon tissue, but only in conjunction with vascular bundles or proconducting elements and in a much smaller size range.

Figure 2 is a low-magnification micrograph of empty cells from peanut cotyledon tissue taken in an SEM. These cells were taken from a similar region to those shown in Figure 1. The cellular contents had been digested out so only the cell walls remained. Microscopy of thin sections did not reveal the intricate three-dimensional nature of the wall sculpturing shown with such clarity with the SEM

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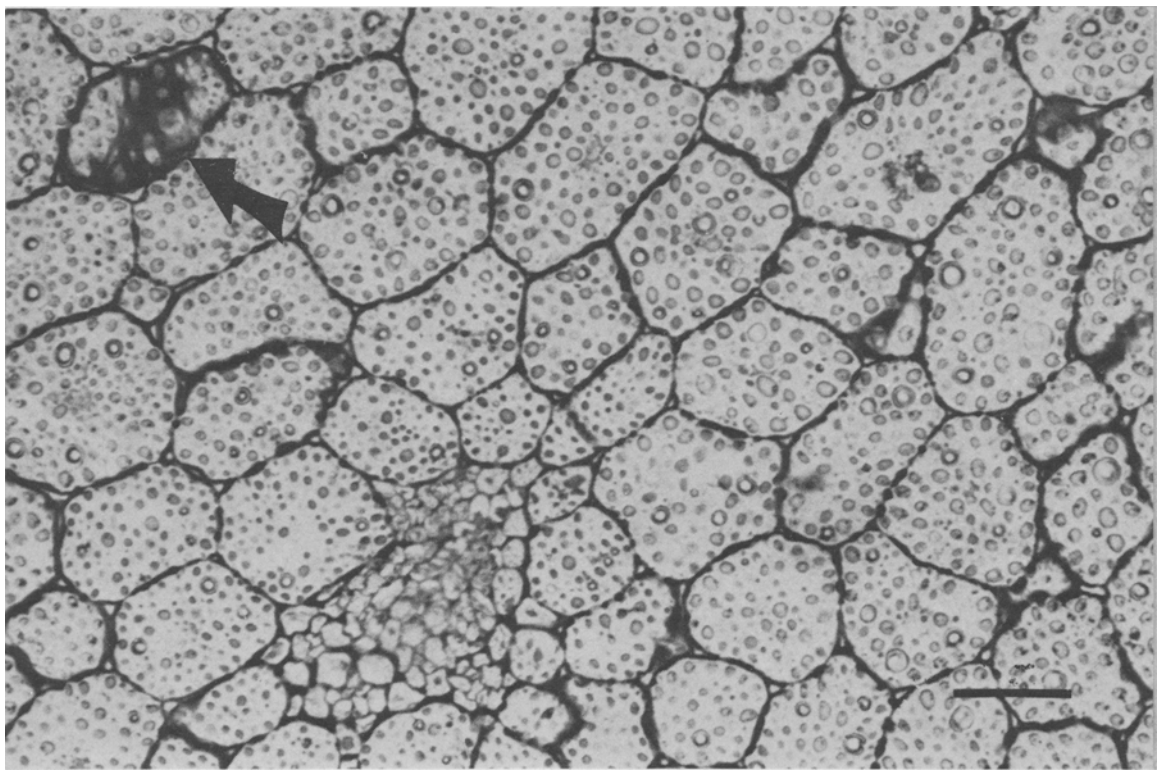

FIG. 1. A light micrograph of a 1.0-1.5 $\mu$m section of peanut cotyledon tissue stained with toluidine blue. Note the bizarre formation pointed out by the arrow. This formation is a tangential section taken right at the edge of the cell; examination of other cell walls shows that there are numerous pits in these cotyledon cell walls. Line equals 50 $\mu$.

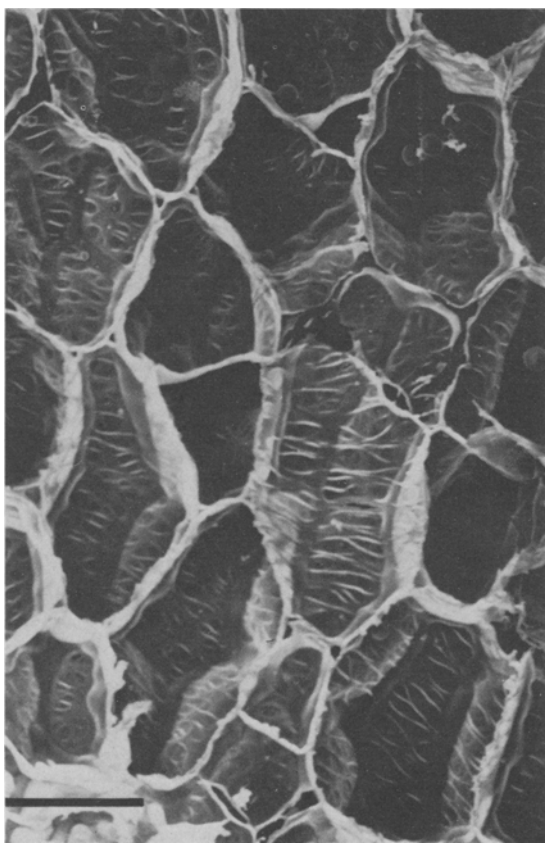

FIG. 2. A low magnification scanning electron micrograph of cell walls of parenchyma tissue from a peanut cotyledon. The cellular contents were digested away with enzymes to allow visualization of the walls. Note the unusual scalariform nature of the pits. Some of the pits are enormous, ranging in size up to 10 $\mu$ and more in diameter. Line equals 30 $\mu$m.

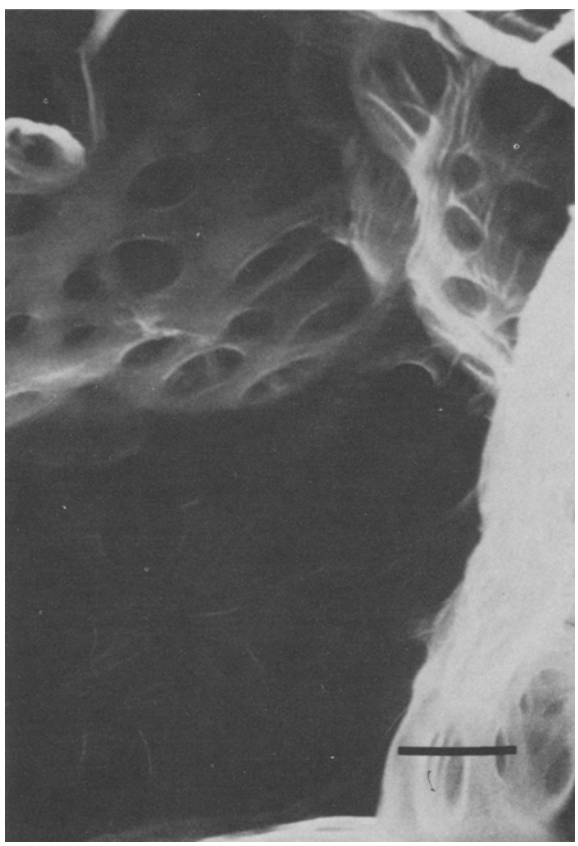

FIG. 3. A slightly higher magnification scanning electron micrograph of the cell walls showing the unusual nature of these pits. Line equals 10 $\mu$m.

(Fig. 3). This type of cell-wall morphology on storage parenchyma cells was surprising.

These results should not have been surprising, however, because micrographs of peanut cotyledon tissue clearly showed that there are pits in the cell walls. The three-dimensional aspect of these pits could have been reconstructed with serial sections. Perhaps Bagley et al. (5) confused the issue when they stated that the pits appeared after germination and that none were seen in resting seeds. The seeds used by us and those used by Neucere and Hensarling (6) were resting seeds and both show pits in the cell walls.

One of the thoughts that occurred to us was that these indentations and sculpturings in the cell wall might impart structural strength, not unlike a keystone arch. To test this hypothesis, we removed the cellular contents from sections of peanut cotyledon tissue as described in Methods and Materials and tested their resistence to crushing in an Instron compression cell. The defatted and enzyme-digested specimen resembled pieces of styrofoam; they were white and very light in weight. To check if, indeed, we had removed the cellular contents, one of the specimen pieces was cut in half with a razor blade and viewed in the SEM. The results were almost identical with that described in the previous paragraph (except that almost all of the cells in this experiment contained starch granules, whereas they occurred rather infrequently in the specimen that was cut prior to defatting and enzyme treatment). Obviously, solutions of lipids in hexane extracted during defatting and pronase degradation products were able to diffuse through the cell walls leaving only the starch granules behind.

The results of the crushing test were inconclusive. The data for both easy-to-press and difficult-to-press peanuts varied and there was no clear difference in their respective resistance to crushing. However, the pressure required to crush the samples to the 80% level for both of these specimens was extremely light, in the range of several pounds per square inch, and not in the hundreds of pounds per square inch required to express oil from the seeds. We conclude, therefore, that the cell walls as structural elements lend very little to the resistance to crushing.

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