Structure and Mineral Composition of Cereal Aleurone Cells as Shown by Scanning Electron Microscopy[1]

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ABSTRACT

Scanning electron microscopy revealed basic similarities in structures of aleurone cells in barley, oats, rye, and wheat. The cell wall(s), the plasmalemma, and the interior of the aleurone cell wall were investigated. The aleurone grains are embedded in a network, which occupies the intergrain spaces. Various organelles and protrusions are confluent with the surface of the aleurone grain. Aleurone grains contain substantially greater quantities of mineral components than the aleurone cell wall. Relative concentrations of mineral components point to the presence of a potassium-magnesium, rather than a calcium-magnesium, salt of phytic acid as the main form of phosphorus in the aleurone grains of barley.

The fine structure of aleurone cells in wheat was described by Buttrose (1) and in barley by Jones (2). Those studies used conventional techniques of fixation, embedding, and sectioning that may induce structural deformations. In addition,

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thin sections prepared for electron microscopy do not expose face views of membranes. To overcome some of these difficulties, Buttrose (3) used freeze-etching. Scanning electron microscopy is uniquely suited to study surfaces of biological materials. The technique also can provide a picture of the spatial arrangement and distribution of organelles in the aleurone cell. Such a picture could not be obtained through previously employed techniques.

A precise way to determine composition of microstructures is by identifying, in an electron microprobe, X-rays emitted from the specimen on impact of the primary beam in the scanning electron microscope. Energies of the emitted X-rays can be identified with a solid-crystal detector and a multichannel analyzer. The detector can be placed close to the specimen to collect many X-rays; it is virtually 100% efficient for X-rays up to 20 keV. This detector is well suited to low probe currents of the scanning electron microscope and can be attached to it without limiting the other uses of the instrument.

The purpose of this investigation was to determine the structure and composition of aleurone cells by scanning electron microscopy and X-ray analysis. Aleurone cells are implicated in the incorporation of amino acids into proteins (4), and in several processes that occur during the modification of cereal grains in malting or mobilization of reserve materials in the endosperm during germination. Consequently, there is great interest in the structure of the aleurone cell, especially as a relation is indicated between the structure, composition, and function of the cell.

MATERIALS AND METHODS

Structural Studies

Longitudinal and cross-sections of mature barley (*Hordeum vulgare* L., cv. Larker), oat (*Avena sativa* L., cv. Orbit), wheat (*Triticum aestivum* L., cv. Triumph), and rye (*Secale cereale* L., cv. Elbon) were mounted on circular (diameter, 9 mm.) aluminum specimen holders with an adhesive, and coated with a 200- to 300-Å gold layer. The specimens were examined in a Cambridge Stereoscan[3] microscope at 20 kv.

Mineral Composition

Transverse sections, about 20 μ thick for electron microprobe analysis, were cut on a microtome with a glass knife from about the midpoint of the kernel of mature barley (*H. vulgare* L., cv. Larker and Conquest). The sections were mounted on circular graphite holders with an adhesive. Uncoated specimens were examined on a Jeol JSM-U3[3] scanning electron microscope equipped with an energy-dispersive X-ray analyzer using an accelerating voltage of 15 kv., -7° tilt, 5×10^{-12} amp. specimen current, and 13 mm. working distance.

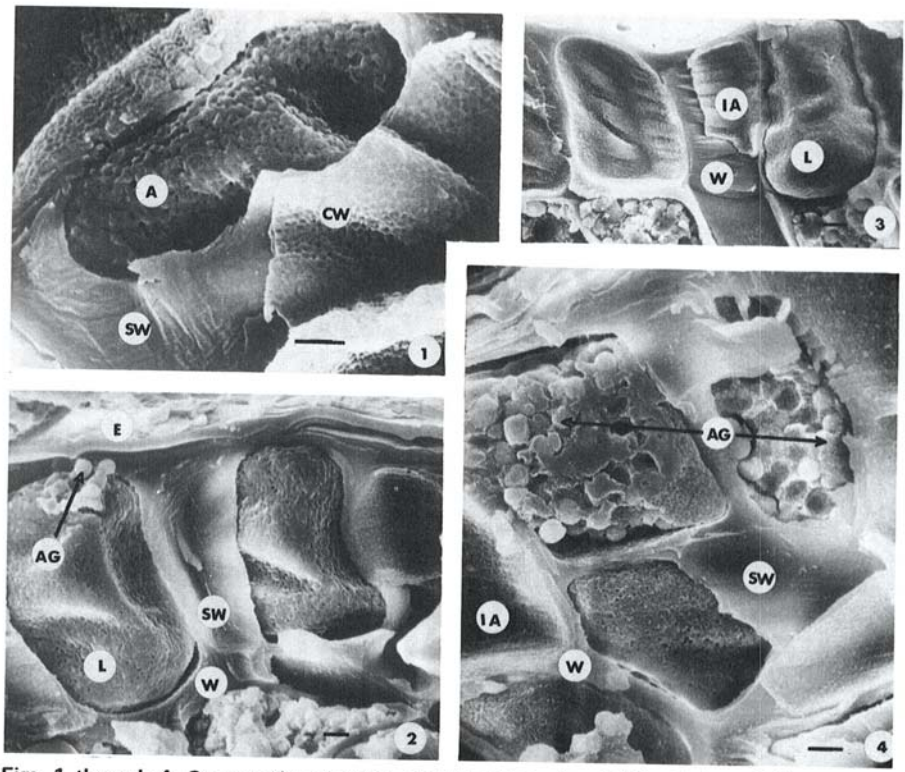
RESULTS AND DISCUSSION

The aleurone tissue of cereal grains consists of one (in most species) to three or four (in barley) cell layers that surround the starchy endosperm. The size and shape

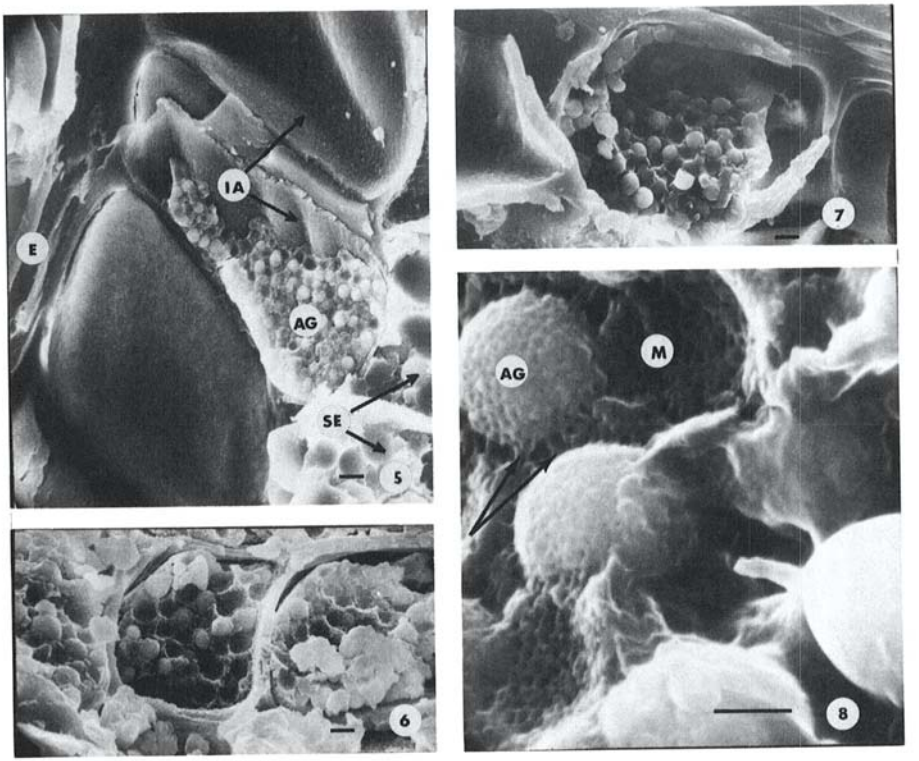
[3]Mention of a trademark name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

of the aleurone cells are not uniform throughout the whole grain, but certain basic characteristics are common to all studied cereals and to all parts of the grain.

The distance between adjacent aleurone cell contents is about 3 μ. A middle lamella separates the individual cell walls. The aleurone cell wall in oats is shown in Fig. 1. Examination by scanning electron microscopy indicates, tentatively, the presence of a primary wall and a fibrillar secondary wall. Positive identification of the two cell wall structures (as well as of the plasmalemma) would require confirmation by optical microscopy and transmission electron microscopy. A cell wall, covering part of two aleurone cells, is shown in Fig. 2. The contents of the two cells form well-defined units, which in the mature grain are separated from the cell wall. The surface of the above units is irregular and pitted and appears to be a membrane or plasmalemma, rather than an interface effect. The interior surface of the aleurone cell wall is comparable in appearance to that of the plasmalemma (Figs. 3 and 4).



Figs. 1 through 4. Cross-section through aleurone layer of oat (Figs. 1, 2, and 3) and barley (Fig. 4) kernel. Fig. 1 shows cell walls (CW and SW) and exposed aleurone cell (A). Line = 3 μm. Fig. 2 shows surface of cell wall (SW) section through cell wall (W), plasmalemma (L), and partially exposed contents of an aleurone cell with aleurone grains (AG). Line = 3 μm. Fig. 3 compares cell wall (W), plasmalemma (L), and interior of an aleurone cell (IA). Line = 6 μm. Fig. 4 shows section through cell wall (W), surface of cell wall (SW), interior surface (IA), and two aleurone cells varying in the extent of exposed inside contents and aleurone grains (AG). Line = 3 μm.



Figs. 5 through 8. Cross-section through aleurone cells of rye kernel (Fig. 5), barley (Fig. 6), and wheat (Fig. 7). Fig. 5 shows adjacent pericarp (E) and starchy endosperm (SE) with embedded (or concave) surfaces from starch granules, aleurone grains (AG) in a matrix, and interior surface of aleurone cells (IA). Line = 3 μm. Figs. 6 and 7 show aleurone grains embedded in a matrix. Line = 3 μm. Fig. 8 shows aleurone grains (AG) embedded in a matrix (M); note (arrow) protrusions between adjacent grains and between aleurone grains and matrix. Line = 1 μm.

The contents of aleurone cells are characterized by prominent, spherical aleurone grains. The arrangement of these grains in the aleurone cell, and the relation of that arrangement to the gross structure of the aleurone and the starchy endosperm, are shown in Fig. 5. The cross-section through a rye kernel shows in the central aleurone cell grains embedded in a matrix, several layers of aleurone cell wall, the plasmalemma, interior of exposed aleurone cells, and protein matrix with embedded (or concave surfaces from) starch granules in the starchy endosperm.

The detailed arrangement of aleurone grains within the cell has been the subject of uncertainty. Buttrose (1) found that, in the light microscope, aleurone grains were clearly separated from each other by a bright material, but in the electron microscope, no cytoplasmic substance appeared between the grains. He concluded that the dense material that was shown by light microscopy to surround the aleurone grains was not part of the latter, but belonged to the embedding substance.

Jones (2) compared the arrangement of organelles in dry and in imbibed aleurone cells. In the imbibed state, the cells were completely filled. In the dry state, the same organelles were present, but because of the difference in the degree of hydration, they did not completely fill the cell. Jones (5) also has shown that in aleurone tissue suspended in water and centrifuged at high speeds, there was a stratification and separation of aleurone grains from spherosomes and other organelles.

Scanning electron microscopy of aleurone cells indicates that the grains are embedded in a matrix, which fills the cells (Figs. 6 and 7). At high magnification (Fig. 8), grains in the dry seed are embedded in a porous network. Numerous organelles, including spherosomes, can be seen on the outer surface of the aleurone grains. After imbibition, the aleurone grains and the network expand and fill the interior of the cells (5,6).

Jones (2) found no indication of fusion of aleurone grains, despite their proximity. According to Paleg and Hyde (7), gibberellic acid-treated aleurone cells showed enlargement, fusion, and formation of vacuole-like areas within the aleurone grains. Scanning electron microscopy (Fig. 8) shows organelles that are confluent with the surface of the aleurone grain. There are numerous cylindrical protrusions (about 0.15 μ long and 0.05 μ thick) on the surface of the grain (up to 2 μ in diameter). But it has not been possible to establish whether they actually form links between grains or between the grains and the embedding matrix. It is possible that the protrusions are actually part of the embedding matrix that covers the grains. Ory and co-workers (8,9) isolated aleurone grains (protein bodies) by centrifugation at 20,000 $\times$ g. Electron microscopy showed that at those speeds various organelles were attached to the periphery of the grains, and enzymatic activities of the preparations were retained.

According to Jones (5), centrifugation at less than 30,000 $\times$ g yielded limited stratification and no measurable loss in gibberellic acid-stimulted α-amylase production. Centrifugation at more than 30,000 $\times$ g enhanced stratification and reduced the response to gibberellic acid. At very high speeds, an irreversible disruption of the porous matrix in which the aleurone grains are embedded might occur.

Information on the mineral composition of structural components in the aleurone cells of barley is both limited and indirect. According to Jacobsen et al. (10), aleurone grains of barley contain two types of inclusions: a) globoids with globoid cavities; the globoids stain positively with toluidine blue (presumably because of the presence of phytin) and with lipid stains, and b) protein-carbohydrate bodies, which stain green with toluidine blue. In addition, some free phosphate was found in the globoids, and high concentrations of phosphate were seen around the periphery of the globoid cavity. In addition to the nonspecific staining technique, Jacobsen et al. (10) presented highly suggestive, though indirect, evidence for the presence of phytin in the globoids of aleurone cells. Tronier et al. (11) separated the proteins of isolated aleurone grains in barley into two fractions. Most of the phosphorus in barley aleurone grains was in the hordein fractions. The results pointed to the possibility that phytate in barley did not exist in specific areas such as globoids.

Figure 9a is a scanning electron micrograph of a transverse section of the aleurone layer in Conquest barley; a phosphorus K_α X-ray map of the area in Fig.

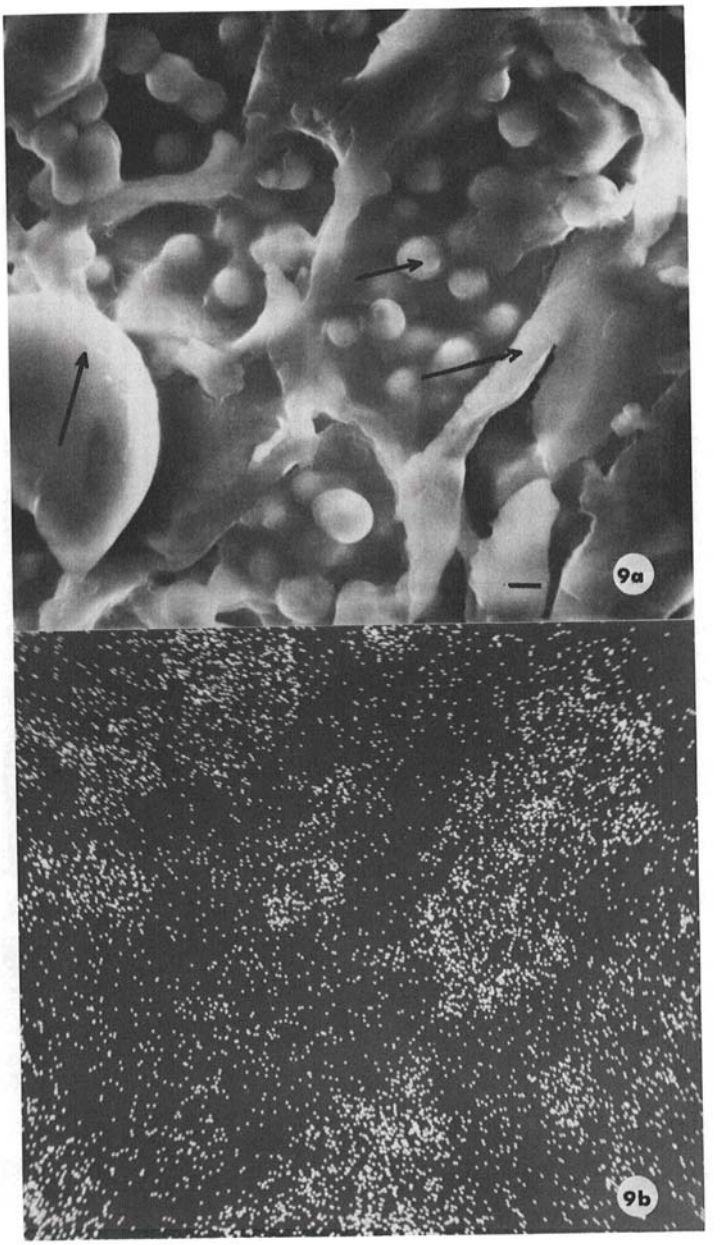


Fig. 9. a, Cross-section through aleurone cells of Conquest barley; arrows show microareas in aleurone grain, aleurone cell wall, and starch granule (from the starchy endosperm) that were selected for X-ray microanalysis. Line = 3 μm. b, X-ray map of the area in Fig. 9a. The higher the concentration of light spots, the higher the concentration of phosphorus. Area was scanned 500 sec.

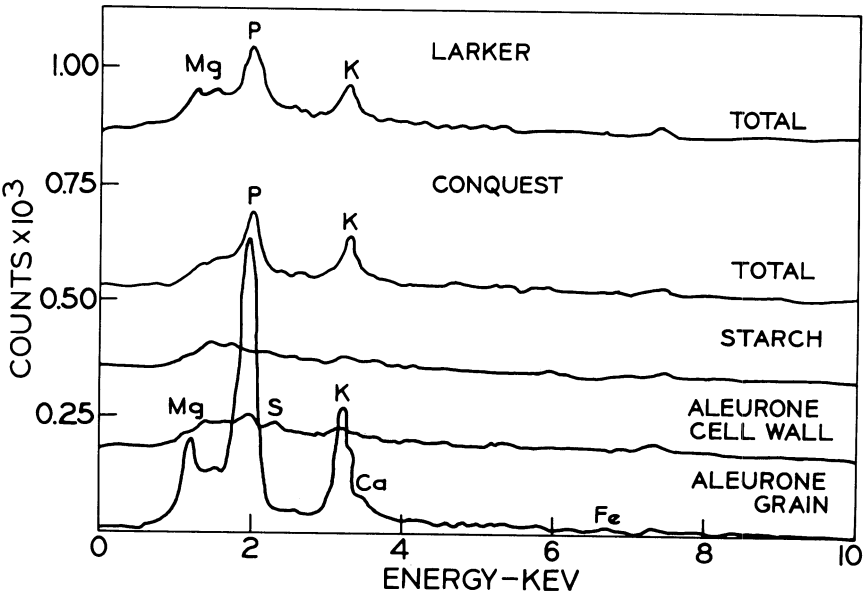


Fig. 10. Recording of X-ray spectra in selected microsections of aleurone grains, aleurone cell wall, and starchy endosperm of Conquest and Larker barleys. Elements present in relatively high concentrations are identified. Areas under the peaks represent counts × 10^3/200 sec. (Graphs of individual samples are shifted for easier identification.)

9a is shown in Fig. 9b. It is clear that the aleurone grains are the sites of relatively high phosphorus concentration, and that there is little phosphorus in the cell walls. These findings are confirmed by scanning the whole area or selected areas (about 1 μ in diameter) in Fig. 9a. The selected areas included sections of the aleurone grains, cell wall, and starch granule. The graphical recording of the X-ray spectra is given in Fig. 10; individual elements are identified according to the energy of emitted X-rays, the amounts of each element present are related to the areas under the peaks (Table I). The starch granule (presumably from the starchy endosperm) contains very low concentrations of minerals. Both the aleurone cell wall and the aleurone grain contain Mg, P, and K. However, concentrations of those elements in the grain are by far greater than in the cell wall. According to the magnitude of emitted X-ray energy, the cell wall contains some sulfur compounds. No consistent differences in mineral composition of tissues and cell components of Larker and Conquest barleys were apparent.

There was a surprisingly low concentration of calcium in the aleurone grain. The results (Fig. 10 and Table I) indicate that if the phosphorus in the aleurone grains is present primarily in phytate, it is in the form of potassium and magnesium phytate (12) and not the calcium-magnesium salt of inositol hexaphosphoric acid (phytic acid) (2,10). In many seeds, the salts of phytic acid are the principal source of phosphorus for phosphate-requiring metabolic reactions (12). The fact that calcium may not be bound to phytic acid seems to be significant, as calcium ions promote the accumulation of amylase by isolated barley endosperm layers in response to gibberellic acid (13) and enhance synthesis and stability of some α-amylase

TABLE I. RELATIVE PEAK AREAS OF ELEMENTS IN THE ALEURONE GRAIN OF CONQUEST BARLEY, AS DETERMINED BY X-RAY MICROANALYSIS

Energy keV.	Identification	Peak Area[a]
1.34	Mg	768
2.03	P	6,654
3.33	K	4,081

[a]After subtraction of background.

isoenzymes (14). Goodwin and Carr (15) have recently shown, on the basis of metal-ion requirements, that the lag phase in the induction of α-amylase synthesis in aleurone layers by gibberellic acid can be divided into two parts. In the first there is a requirement for iron, in the second, for externally added calcium. X-ray analysis indicates that both metals are present in very low concentrations in the aleurone layer.

Acknowledgments

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