THE RICE SCUTELLUM. II. A COMPARISON OF SCUTELLAR AND ALEURONE ELECTRON-DENSE PARTICLES BY TRANSMISSION ELECTRON MICROSCOPY INCLUDING ENERGY-DISPERSIVE X-RAY ANALYSIS

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ABSTRACT

Observation of scutellar cells with a transmission electron microscope revealed that the morphological appearance of the scutellar cells is quite similar to that of the aleurone cells. Protein bodies and spherosomes are present throughout the scutellar cells. The chemical composition of electron-dense inclusions was established by energy-dispersive X-ray analysis, indicating that the inclusions were deposits of magnesium and potassium salts of phytic acid.

The structure of scutellar cells of rice grain has been investigated by scanning electron microscopy, which revealed that many spherical particles were the main component (1). Electron microprobe X-ray analysis also showed similar distribution of minerals such as phosphorus, magnesium, and potassium both in the aleurone layer and in the scutellum of rice (1).

The structure of the scutellum of wheat was studied by Swift *et al.* (2,3) using light and electron microscopy, and they established that the scutellar cells of wheat were characterized by the existence of protein bodies and spherosomes. The conspicuous feature of the protein body of scutellar cells was that high electron-dense inclusions (usually called "globoid" or "globoid crystal") were embedded in the proteinaceous matrix.

These findings suggested that the scutellar cells of rice also contain those particles characterized by high contents of magnesium and potassium salts of phytic acid, and the cells are morphologically similar to those of aleurone.

The goal of this study was to clarify the morphological appearance of the rice scutellar cells and the subcellular particles.

MATERIALS AND METHODS

Rice grains (*Oryza sativa* L. cv. Koshihikari) used for transmission electron microscopical observation were grown at a rice plot. The mature grains were harvested and stored in a desiccator with silica gel at 5° C. Then the rice grains were soaked for 24 hr in water at 0° C and the embryo- and aleurone-containing regions were obtained using a razor. The materials were fixed with 3% glutaraldehyde in phosphate buffer at pH 7 for 2 hr at 0° C and subsequently with 1% osmium tetroxide in the same buffer for 1 hr. After dehydration in graded dilutions of ethyl alcohol, the materials were embedded in epoxy resin (Epikote 812). Sections (0.1 μm) of the embedded materials were cut with an ultramicrotome, Porter-Blum MT-1, with a glass knife. Photographs were taken with a JEM-7-type electron microscope (Japan Electron Optics Laboratory Co., Tokyo).

The elementary analysis in the microrange of the sections of rice grains was carried out with a JEM 100C transmission electron microscope system fitted with an Ortec Model 6200A Multichannel Analyzer. The sections used in this analysis were made according to the same procedure for transmission electron microscopy, except that the sections were 0.3 μm in thickness.

RESULTS AND DISCUSSION

Previous work of our laboratory suggested the occurrence of high-phytin-containing particles in the scutellar cells of rice, when scanning electron microscopy and electron microprobe X-ray analysis were used (1). However, no direct evidence has been presented for the high-phytin-containing bodies believed to be sites of phytin accumulation in the grain.

As shown in Fig. 1, A, the general appearance of the rice scutellar cells in the transmission electron microscope is similar to that of the rice aleurone cells (Fig. 2, A). Scutellar cells are characterized by protein bodies which correspond to aleurone granules in the aleurone cell, and by numerous spherosomes. One or a few high electron-dense inclusions are embedded in the matrix of a proteinaceous particle. These proteinaceous particles, which contain electron-dense inclusions, are seen both in the scutellar epithelial cells and in the scutellar parenchyma cells, although the frequency and size of the electron-dense inclusions are greater in scutellar parenchyma cells than in scutellar epithelial cells. From our knowledge of transmission electron microscopy of aleurone cells of many plant seeds, the high electron-dense inclusions were supposed to be phytin deposits (2,4–6). The highly magnified features of the electron-dense inclusion are shown in Fig. 1, B. The electron-dense inclusion is quite similar to that seen in the proteinaceous matrix of aleurone granules (Fig. 2, B). The surface of the inclusion is apparently enveloped by a thin membranous material, shown by an arrow. In Fig. 1, B, we could find only one kind of electron-dense inclusion. Another electron-dense inclusion composed of protein and carbohydrate [such as that described by Jacobsen *et al.* (4) in the proteinaceous matrix of barley aleurone grain] was not detected in the protein bodies of rice scutellum. The surface of the protein body of the scutellum is covered with spherosomes.

Figure 3, A and B, shows EDX[1] spectra of electron-dense inclusions which appeared in the scutellar parenchyma cell and in the aleurone cell of rice, respectively. The two spectra cannot be differentiated from each other, indicating similar elemental composition; namely, the electron-dense inclusions are composed of phytic acid as the salts of potassium and magnesium. Although phytin is generally reported to be a calcium and magnesium salt of myoinositol hexaphosphoric acid (7), as shown in Fig. 3, A and B, electron-dense areas of rice grain should be composed of a magnesium and potassium salt. This agrees with the chemical composition reported on the isolated particles from rice grains (6). Lott (5) made an EDX-analysis on a globoid crystal section of cotyledon tissue from dry squash seeds and reached the same conclusion—that the globoid crystal was mainly the phytin deposit of a magnesium and potassium salt.

EDX-analysis was also conducted on a protein body which appeared in the endosperm cells of rice. The spectrum of Fig. 3, C is different from that of A and B, indicating that protein bodies in endosperm cells are not the accumulation

[1]EDX: energy-dispersive X-ray.

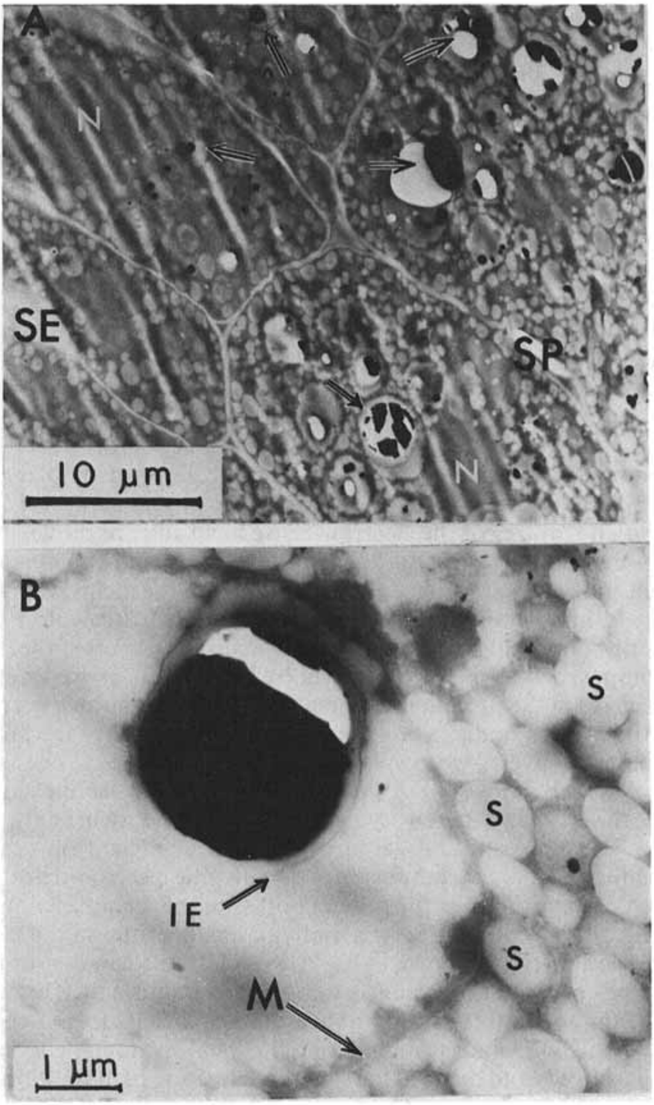

Fig. 1. A) A transmission electron micrograph of scutellar parenchyma (SP) and scutellar epithelium (SE) cells; N, nucleus. High electron-dense inclusions are shown by arrows. B) A high magnification transmission electron micrograph of an electron-dense inclusion embedded in a proteinaceous matrix of a subcellular body of a scutellar cell. S, spherosome; M, membrane of proteinaceous body; and IE, inner envelope.

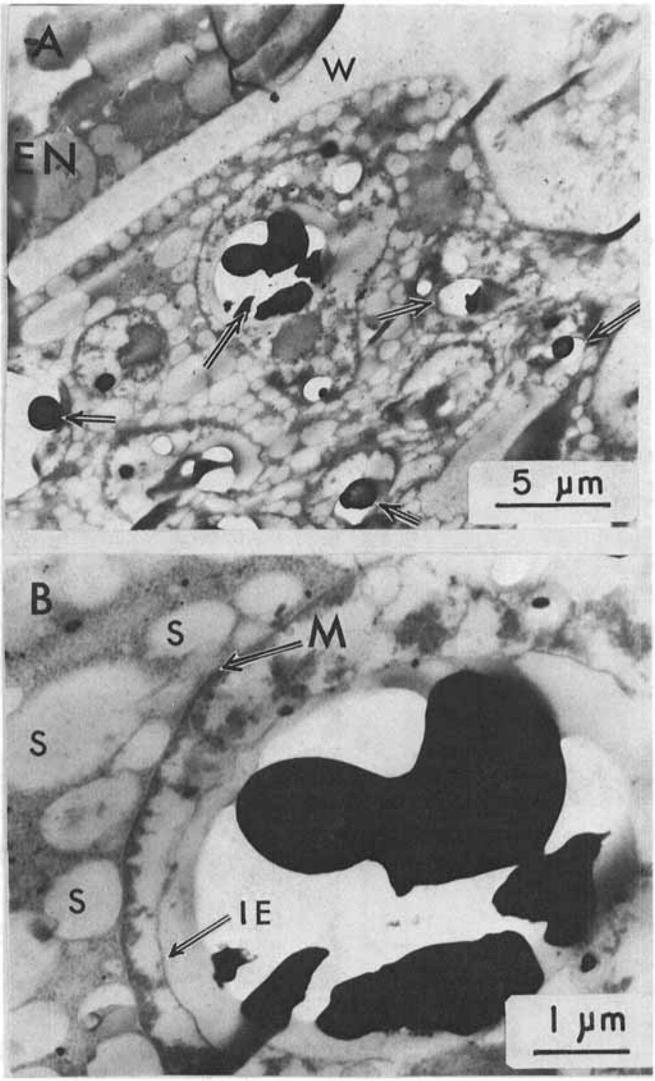

Fig. 2. A) A transmission electron micrograph of an aleurone cell: EN, endosperm; and W, cell wall. High electron-dense inclusions are shown by arrows. B) A high magnification transmission electron micrograph of an electron-dense inclusion embedded in a proteinaceous matrix of a subcellular body of an aleurone cell. S, spherosome; M, membrane of an aleurone granule; and IE, inner envelope.

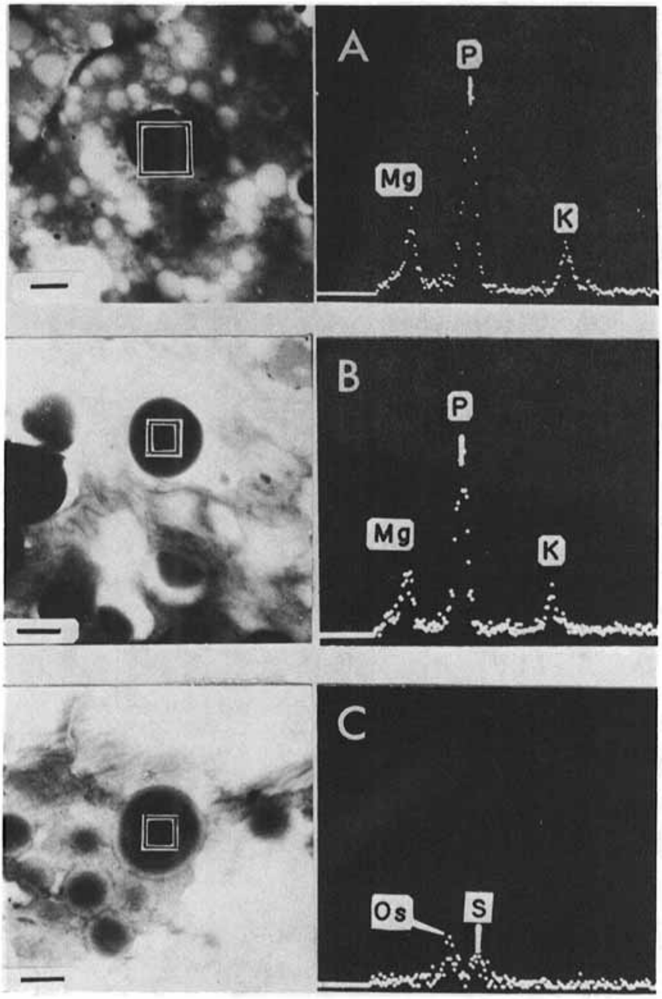

Fig. 3. EDX spectra of electron-dense areas in rice grain. A) EDX spectrum of an electron-dense inclusion embedded in a protein body of scutellum; B) EDX spectrum of an electron-dense inclusion embedded in an aleurone granule and C) EDX spectrum of a protein body in the endosperm of rice grain. The principal emission line of each element used for identification is as follows (in KeV): magnesium 1.253, Kα; phosphorus 2.013, Kα; potassium 3.312, Kα; sulfur 2.307, Kα; osmium 1.914, M. The assayed areas are shown by squares on the left side of the EDX spectrum. Scale bars represent 1 μm.

sites of phytic acid. By contrast, sulfur and osmium were detected. For the sulfur peak, sulfur-containing amino acids such as methionine and cystine may be responsible. However, the osmium peak is caused by OsO_4 fixation.

Transmission electron microscopy and EDX-analysis demonstrated that the protein bodies which appear in scutellar cells are quite similar to aleurone granules and not to protein bodies in endosperm cells. By EDX-analysis, it can be concluded that phytic acid exists mainly as potassium and magnesium salts throughout the tissue of rice grains.

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