

# THE RICE SCUTELLUM: STUDIES BY SCANNING ELECTRON MICROSCOPY AND ELECTRON MICROPROBE X-RAY ANALYSIS

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## ABSTRACT

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Observations of the rice scutellum using a scanning electron microscope indicated the scutellar cell contains many spherical particles which resemble aleurone particles of the aleurone layer. The distribution of P, Mg, and K in the scutellar cell observed by electron

microprobe X-ray analysis is very similar to that in the aleurone cell. Therefore, it is reasonable to conclude that both tissues contain particles characterized by high contents of Mg and K salts of phytic acid.

The scutellum, a part of the embryo of monocotyledonous seeds, lies between the starchy endosperm and the embryonic leaves and roots. It is believed, therefore, that this tissue plays an important part as the pathway along which reserve materials in the endosperm move to the growing axis after germination. In addition, considerable quantities of fat, protein, and carbohydrates are stored within the scutellum (1). Further, some plant hormones (2,3) and hydrolytic enzymes (3,4) are secreted from the scutellum into the endosperm and the aleurone layer.

Ultrastructural studies on wheat scutella performed with the aid of transmission electron microscopy (TEM) revealed that the scutellar cells contain subcellular particles surrounded by numerous lipid droplets (5,6). It has been further established that electron-dense inclusions were embedded in these particles (5,7). These observations demonstrated that the wheat scutellar cells morphologically resembled the aleurone cells.

In this study, the structure of the rice scutellum was examined by scanning electron microscopy (SEM) in order to obtain further information on the scutellar cells of monocotyledonous seeds. Electron microprobe X-ray analysis was also used to survey the distribution of phosphorus (P), magnesium (Mg), and potassium (K) in the tissue. The results obtained show that rice scutellar cells and elemental distribution in the cells are strikingly similar to those of the aleurone cells.

## MATERIALS AND METHODS

### Scanning Electron Microscopy

Rice grains (*Oryza sativa* L. cv. Koshihikari) were obtained from experimental plots. The matured grains were harvested and stored at 5°C for a period which did not exceed 1 year. The grain was cut in half longitudinally through the apex and the base of the scutellum. One piece with half an embryo was adhered to a brass specimen stub using Araldite. Any cracks between the sample and the stub were filled with Dotite (an electrically conductive paste produced by Fujikura Kasei Co., Ltd., Japan). The surface of the sample was coated with carbon followed by gold, and viewed in a JEOL JSM-U-3 SEM.

### Electron Microprobe X-Ray Analysis

The rice grains were cut transversely into equal halves with a razor, and then a half grain with the embryo was embedded in epoxy resin, Epikote 812. They were evacuated at  $10^{-3}$  TORR for 2 hr; then the sample in the epoxy resin was stored in a small gelatin capsule at  $60^{\circ}\text{C}$  and left to harden. The sample was trimmed using a microtome with a glass knife to create a smooth surface for observation.

An electron microprobe X-ray analyzer, Shimadzu-ARL EMX-SM type, was operated at 25 kV for P, K, and Mg, with a sample current of 3 nA, and an X-ray beam at less than 500 Å. The X-ray spectrometer was peaked for the first-order  $K_{\alpha}$  line using ammonium dihydrogen phosphate for P and K, and rubidium acid phosphate for Mg. Secondary electron images were employed to examine the surface of the samples and to select the region for analysis. Qualitative analysis for each element was made by line scanning and two-dimensional scanning.

## RESULTS AND DISCUSSION

Using the light microscope to display the investigated area as shown in Fig. 1, a section  $1\ \mu\text{m}$  thick was made from the surface of the same area of the sample which was subjected to electron microprobe X-ray analysis.

### Scanning Electron Microscopy of the Scutellar Cells

The SEM of the scutellar cells was carried out in the area P-2. About 18 layers of cells are stratified (Fig. 2a). The general appearance of the cells is similar throughout the scutellum. The cells in the center of the scutellum are larger than the cells which border the endosperm or the coleoptile. Scutellar cells contain

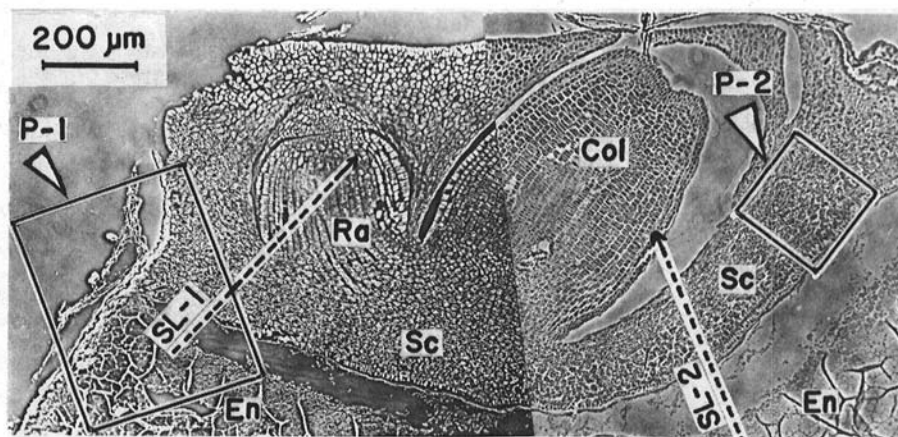


Fig. 1. Phase-contrast light micrograph of embryo-containing region of rice grain showing the investigated areas—a thin section ( $1\ \mu\text{m}$ ) taken from the surface of the sample for electron microprobe X-ray analysis. P-1 = the area investigated by electron microprobe X-ray analysis, and P-2 = a similar area investigated by SEM. The results are in Fig. 2, a and b. SL-1,2 = the loci along which line scanings for P( $K_{\alpha}$ ), Mg( $K_{\alpha}$ ), and K( $K_{\alpha}$ ) were done. Sc, Col, Ra, and En are abbreviations of scutellum, coleoptile, radicle, and endosperm, respectively. The same abbreviations are used in Figs. 2-4.

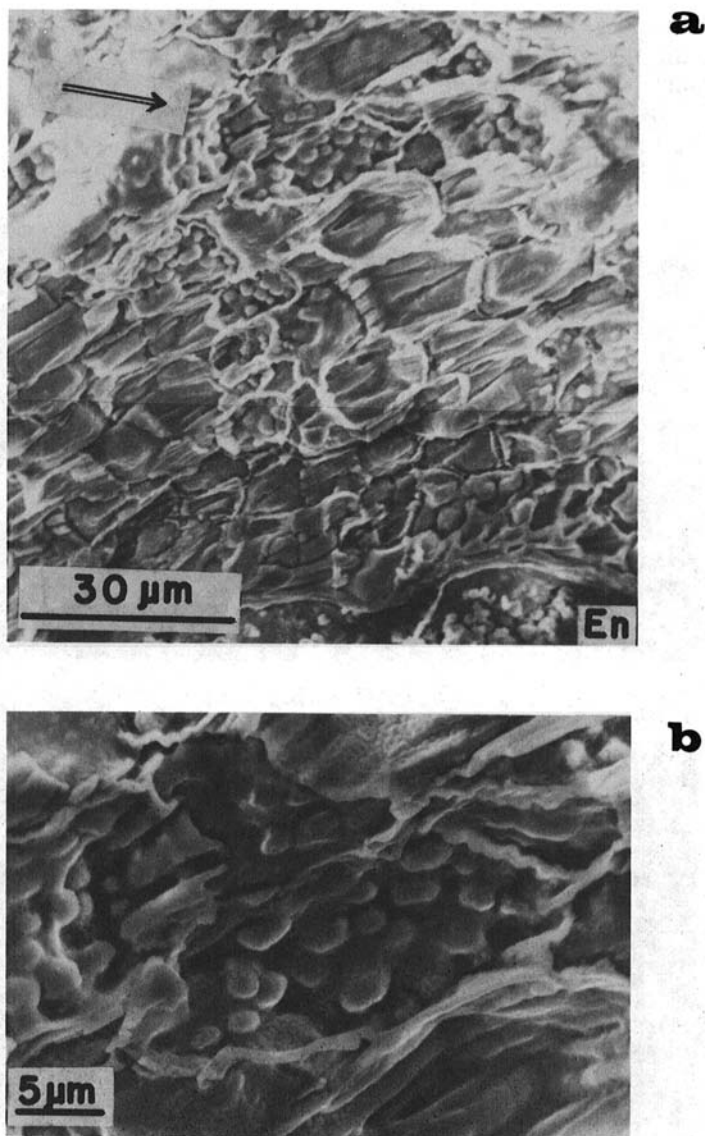


Fig. 2. SEM micrographs of rice scutellum: a = a SEM (the area corresponds to P-2 in Fig. 1); b = highly magnified micrograph of a part shown by an arrow in Fig. 2a. Many rounded inclusions are seen in the cell.

many rounded particles, as shown in Fig. 2b. These particles are around 2–3  $\mu\text{m}$  in diameter and seem to be covered with a membranous coat. As far as the SEM observations were concerned, the appearance of the scutellar cells and the shape of the spherical particles in them are strikingly similar to those of aleurone cells and aleurone particles, respectively (8).

#### Electron Microprobe X-Ray Analysis of Phosphorus, Magnesium, and Potassium

Two-dimensional scans of Mg, P, and K were carried out at the base of the scutellum (P-1 in Fig. 1). Line scanning was also employed at the areas of SL-1 and SL-2 in Fig. 1. Figure 3a is a light micrograph corresponding to the area P-1. Figures 3b, c, and d are two-dimensional scans of Mg( $\text{K}_\alpha$ ), P( $\text{K}_\alpha$ ), and K( $\text{K}_\alpha$ ),

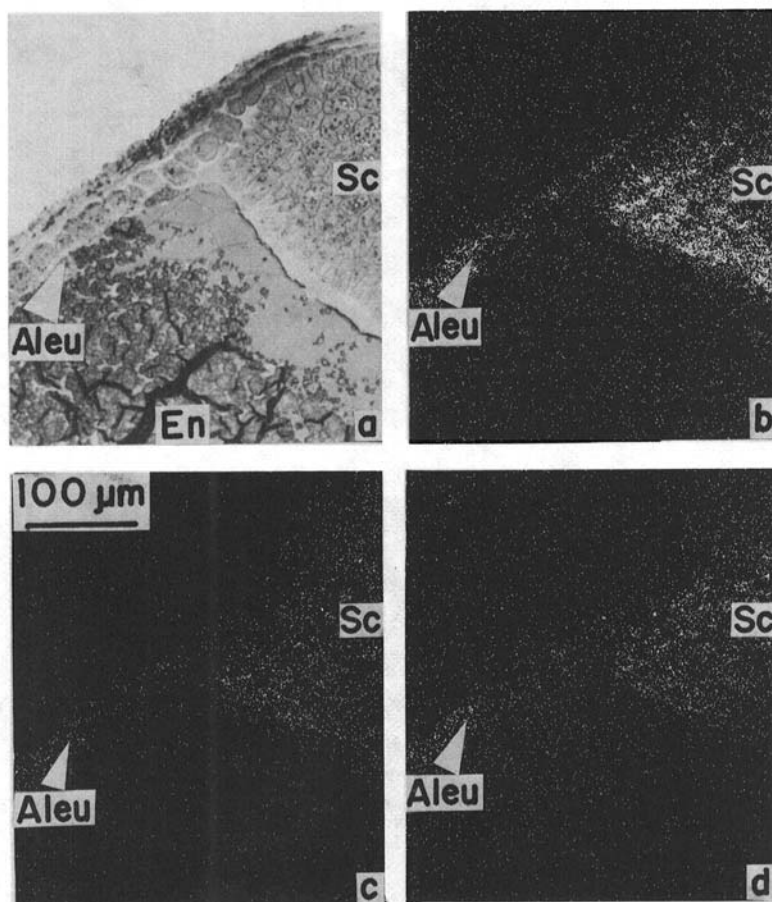


Fig. 3. Secondary electron image and X-ray dot images—P, Mg, and K of the area P-1 in Fig. 1: a = a light micrograph showing aleurone layer (Aleu), scutellum, and endosperm. Iodine staining was employed. b, c, and d = X-ray dot mapping scans of Mg( $\text{K}_\alpha$ ), P( $\text{K}_\alpha$ ), and K( $\text{K}_\alpha$ ) radiations, respectively.

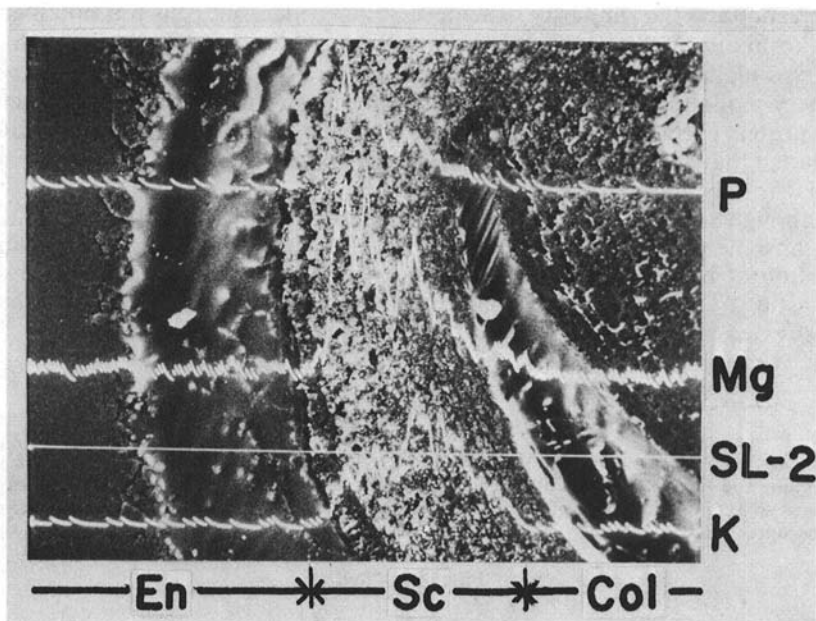
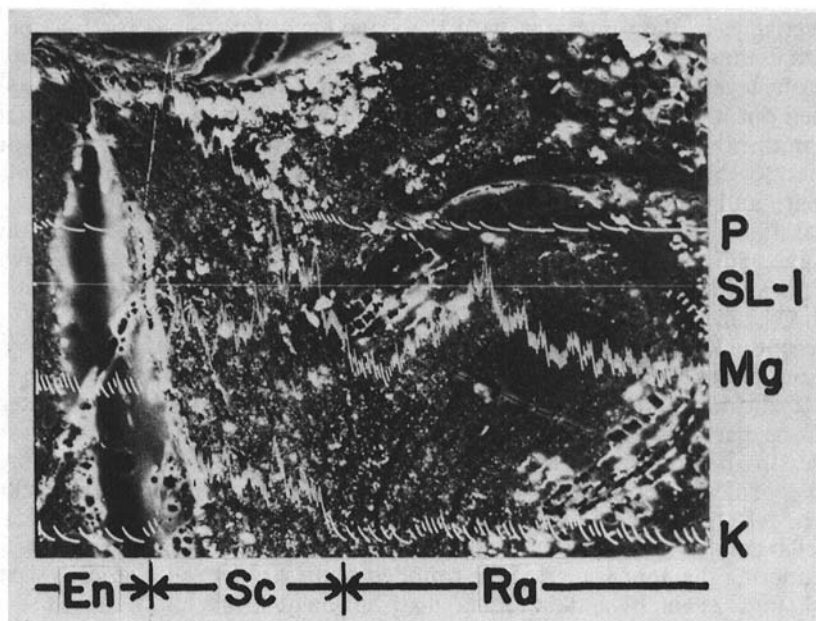


Fig. 4. Secondary electron images of a rice scutellum cross-section, with X-ray line scans for P, Mg, and K. P, Mg, and K are line scans of  $K(K_{\alpha})$ ,  $Mg(K_{\alpha})$ , and  $P(K_{\alpha})$  radiations, respectively. Line scans were carried out from left to right along the lines SL-1 for a and SL-2 for b as shown in Fig. 1.

respectively, in the same area with the same magnification as for Fig. 3a. As shown in these figures, all three elements are condensed in the scutellum and the aleurone layer. Although the quantitative determination of each element was not carried out, the content of each element in the scutellum seems to be similar to that in the aleurone layer. The distribution patterns of these elements are quite similar to those of the aleurone layer (9). The contents of these elements in pericarp and endosperm are very low. The distribution of these elements at the apical side of the scutellum was also examined and similar results were obtained. The line scans of these elements on the lines SL-1 and SL-2 of Fig. 1 are shown in Figs. 4a and b. These results also show that the concentrations of these elements are higher in the scutellum than in the endosperm.

Recently, we isolated electron-dense inclusions from rice aleurone tissue by the aqueous polymer two-phase system, and established that these particles were Mg and K salts of phytic acid (10). The electron-dense inclusions were restricted to aleurone particles and were never found in other organelles such as protein bodies in the endosperm (8). The electron-dense inclusion was, therefore, a marker of aleurone tissue. However, in all TEM micrographs of wheat scutellum, similar electron-dense inclusions have been observed, indicating that the scutellar cells cannot be differentiated from the aleurone cells in this respect (5,7). Considering the appearance of the rounded bodies in rice scutellar cells seen by SEM (Figs. 2a and b), and the similar distribution of P, Mg, and K in the tissue to that in aleurone tissue (Fig. 3), it is concluded that the elemental composition of the rice scutellar cells is similar to that of the rice aleurone cells. Another fact which supports the similarity of scutellar cells to aleurone cells was obtained by Horikoshi and Morita (11). Morita and Yoshida purified  $\gamma$ -globulin from rice embryos including scutellum (12). Horikoshi and Morita prepared an antibody of the  $\gamma$ -globulin using rabbit. This antibody showed an immunocross-reaction with protein extracted from isolated rice aleurone particles (8). This observation suggested that aleurone particles contained a protein which was identical or very similar to the  $\gamma$ -globulin of the rice embryo.

Although scutellar cells (diploid) and aleurone cells (triploid) differ in their chromosome numbers (13), the similar roles of scutellum and aleurone tissue in secretion of hydrolytic enzymes and the similar outward appearance are very interesting. The roles of the similar particles found in both tissues are being investigated in detail.

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