Composition and Endosperm Structure of Developing and Mature Rice Kernel[1]

AURORA R. DEL ROSARIO, VIVIAN P. BRIONES, AMANDA J. VIDAL, and **BIENVENIDO O. JULIANO**[2], The International Rice Research Institute, Los Baños, Laguna, Philippines

ABSTRACT

Analyses were made on the kernel of *Oryza sativa* L. (variety IR8) during development, starting a few days after flowering and continuing until maturity. Maximum length of the caryopsis was attained first, followed by width, and then thickness. The amounts per kernel of starch and protein increased, whereas that of water decreased. Histochemical examination of the endosperm showed the presence of compound starch granules in the 4-day caryopsis, whereas protein bodies were observed distinctly in the 7-day sample. The white-belly portion of the caryopsis corresponded to layers of cells with loose packing of starch granules dispersed in a proteinaceous material. Loose arrangement of cell contents characterized similar opaque portions of white-core and crumbly samples of rice.

Various aspects of the structure (1–4) and chemical changes (5,6) occurring in the developing rice kernel have been studied previously. However, information was required concerning changes not only in starch and protein components but also in the associated endosperm structure during development, as bases for understanding the physicochemical changes during kernel storage (7) and for more detailed biochemical investigations (8,9).

This paper describes the changes in starch, Kjeldahl protein, moisture, and endosperm morphology during development of the kernel of rice, variety IR8. The morphological characteristics of white-belly of IR8 endosperm and other opaque samples were also studied.

MATERIALS AND METHODS

Samples of developing rice kernels were obtained from a 1966 dry-season multiplication plot of IR8 on the Institute farm, fertilized with 100 kg./ha. N. The upper one-third of representative panicles were gathered 4, 7, 11, 14, 21, 28, and 39 days after flowering. The mean flowering date was May 12, 1966. The panicles were individually classified in the laboratory, hand-threshed, and stored in air-tight bottles at –20°C. until analyzed. Samples for histological studies were fixed immediately.

The weight of 100 fresh kernels was determined in duplicate. Observations were made on the appearance of the caryopsis (brown rice). Mean length, width, and thickness of 10 caryopses were determined with a vernier caliper.

Moisture content of rough rice was determined by the two-stage oven method (10). Part of the air-dried (40°C.) ground rice was analyzed for Kjeldahl nitrogen (10) from which Kjeldahl protein was calculated (N $\times$ 5.95). Starch was extracted from rice powder with 2*N* sodium hydroxide with gentle heating until the starch dissolved. The suspension was cooled, neutralized with 1*N* sulfuric acid, and filtered through a coarse sintered-glass filter. An aliquot of this starch filtrate was subjected to phenol-sulfuric acid

[1]Contribution from The International Rice Research Institute, Los Baños, Laguna, Philippines. Mail address: Manila Hotel, Manila, Philippines.

[2]Research Assistants and Associate Chemist, respectively.

assay (11) with glucose as standard, and starch content was calculated (glucose × 0.90).

Rice kernels were dehulled by hand and the middle third of the caryopsis was excised with a razor blade from the rest of the sample for histological studies. The caryopsis was then fixed for 3 days in one of the following solutions: 10% glycerol, formalin-acetic acid-ethanol, Zirkle's modification of Erliki's fluid, or Carnoy's solution (12). The fixed sample was then washed in running water for at least 5 hr. and dehydrated under reduced pressure in a graded series of ethanol (5, 10, 30, 50, 70, and 100%). It was soaked under reduced pressure three times for 4-day periods in a 4:1 (v./v.) mixture of butyl-methyl methacrylate, the third change containing 1% benzoyl peroxide as catalyst. The samples were transferred into gelatin capsules with the methacrylate mixture, which was then polymerized at 60°C. for 12 hr. The methacrylate block was then trimmed, mounted in paraffin (12), and cut with a rotary microtome into sections 2–5 μ thick. The paraffin and methacrylate embedding materials were removed by soaking the sections in xylene and toluene. Stains used on the sections included mercuric chloride-bromphenol blue (13) for protein, fast-green FCF-iodine (14) for cellulose cell walls and starch and hematoxylin (15) regardless of fixative used. Aside from IR8 caryopsis, histological preparations were also made of developing Malagkit Sungsong and Acc. No. 9795 (PI 215936 × CI 9214) and mature caryopses of BPI-76, Century Patna 231, Taichung (Native) 1, and "crumbly" line No. 7154 (CI 1239 × Early Prolific 1943 Cross 446).

Photomicrographs were taken with Kodak Panatomic X film in a Leitz SM phase-contrast microscope with a Leica camera micro attachment.

RESULTS AND DISCUSSION

The rice caryopsis developed much faster in the longitudinal than in the transverse axis. It had attained full length in the 4-day kernel, maximum width with the 14-day kernel, and maximum thickness with the 21-day sample (Fig. 1). Similar results were reported for the developing caryopsis of a Japanese rice variety (16).

Water content of the kernel decreased progressively during development, in terms of both concentration and amount per kernel (Fig. 1). Moisture content per kernel was highest in the 7-day sample. Starch and protein content per kernel increased during maturation. Kernel weight increased and reached optimum value in the 28-day sample. The 28- and 39-day kernels had practically the same composition. The increase in kernel dry matter was due mainly to deposition of starch and, to a lesser extent, protein. In terms of concentration, protein content was highest in the 21-day kernel. Similar curves for dry matter and total nitrogen have been reported for the developing kernel of a Japanese rice variety (17).

Rapid morphological changes in the endosperm cells were observed during kernel development. The aleurone layer cells were nucleated in the 4-day kernel and were two to three cells thick in the dorsal area. The layer was only one cell thick in the rest, including the ventral area, as shown by hematoxylin staining (Fig. 2, a). The cells appeared rectangular on the ventral side and polygonal on the dorsal side.

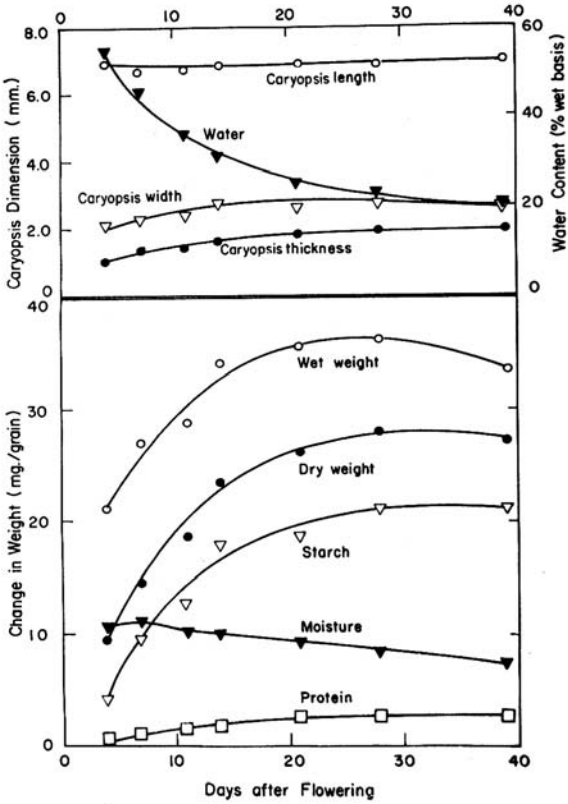


Fig. 1. Changes in dimensions of caryopsis and moisture, weight, starch, and protein of developing IR8 rice kernel.

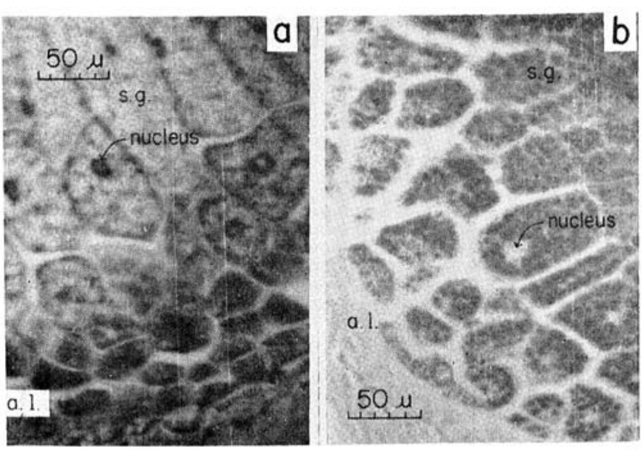


Fig. 2. Cross-section of the ventral portion of 4-day IR8 endosperm: a.l., aleurone layer; s.g., starch granules. Stain: a) hematoxylin; b) fast green-iodine.

The aleurone layer was already fully developed in the 7-day kernel. The cells were filled completely with aleurone grains and the nucleus could not be detected. Cho (1) similarly reported the aleurone layer of Japanese rice to be completed morphologically on the seventh day after flowering.

The number of aleurone cell layers must be a varietal characteristic, since various workers (1,3,4) have reported different numbers of aleurone layers, ranging from one to five in different varieties. In addition, the dorsal side always had a thicker aleurone layer than the ventral area. Japanese rice has been claimed to have more aleurone cells than tropical *indica* rice (1). Century Patna 231, a *japonica* × *indica* hybrid, had three to five cell layers in the dorsal portion of the aleurone. Juliano and Aldama (3) reported that the aleurone layer of a Philippine rice variety varies from one to as many as five layers of cells, whereas Santos (4), for another variety, reported only a single cell layer.

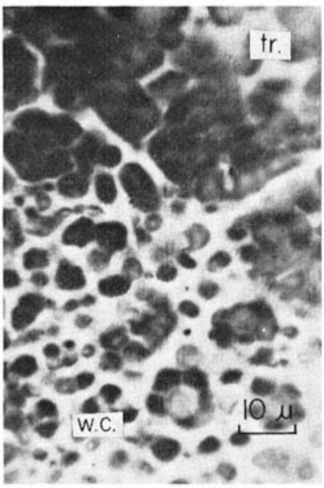
The cells of the starchy endosperm had thinner walls than the aleurone cells. The cells along the lateral axis were isodiametric and elongated along the dorsiventral line, except at the ventral and dorsal regions adjacent to the aleurone layer. In these regions, radially flattened cells were noted: three to four cells thick at the ventral and one to two cells thick at the dorsal side. In the 4-day kernel, the cell contained mainly compound starch granules which were loosely packed, especially in these radially flattened peripheral cells (Fig. 2). The 4-day kernel already contained 4 mg. of starch (Fig. 1). Spaces between the starch granules stained for protein, but very few protein particles (18,19) could be distinguished, particularly in the center of the endosperm. The peripheral cells adjacent to the aleurone layers were still nucleated. The cell walls stained green with fast-green. Cho (1) reported the first appearance of starch in the 5-day kernel of a Japanese rice sample, and Sato (20) also reported its initial occurrence 4 to 5 days after fertilization.

By staining the 7-day kernel with either hematoxylin or bromphenol blue, protein bodies were shown distinctly to be in all endosperm cells. The ventral portion had a greater protein-staining area and a correspondingly smaller starch-iodine staining area than the dorsal portion. These granular structures were classified as protein bodies on the basis of their relative abundance and distribution in the kernel. The protein:starch ratio of the 7-day kernel was 1:7 (Fig. 1). The peripheral cells had more protein bodies than those in the center of the endosperm. Since starch granules were noted in quantity in the 4-day caryopsis, whereas numerous protein bodies were observed only in the 7-day sample, starch synthesis must precede a protein-body synthesis in the developing rice caryopsis. The same sequence of macromolecule syntheses was observed by Jennings *et al.* (21) for developing wheat kernel.

The compound starch granules were more polygonal in shape and more compact in arrangement in the 11-day kernel than in the 7-day endosperm. The protein-staining area appeared to have decreased, probably owing to pressure of the growing starch granules. This corresponded to a greater increase in starch content than in kernel protein content (Fig. 1).

The compact arrangement of the starch was more evident in the 14-day kernel, where iodine staining was quite uniform throughout each cell and the outline of the compound starch granules was less evident, especially at the center of the endosperm. In the 14-day kernel, about 80% of the starch in the mature kernel had already been synthesized (Fig. 1).

In the 21-day kernel, the radially flattened cell layer was three cells thick in the ventral area, and not more than one cell layer remained in the dorsal area. Starch granules were still loosely packed, in contrast to the compact arrangement in the adjacent translucent portion (Fig. 3). Many simple starch granules were noted in these cells with loose starch packing, particularly on higher magnification of an iodine-stained section. Similar observations were noted in the 28-day kernel. Little and Dawson (22) noted that peripheral cells adjacent to the aleurone layer of rice caryopses were radially flattened.



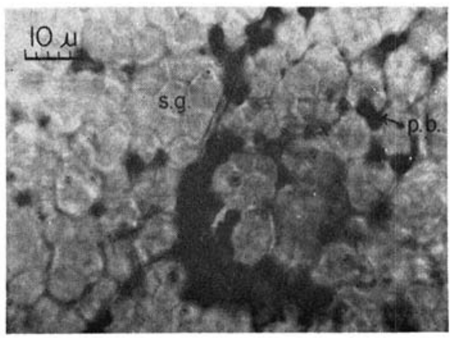


Fig. 3 (left). Cross-section of the ventral portion of 21-day IR8 endosperm stained with fast green-iodine, showing white-core (w.c.) and translucent (tr.) areas.

Fig. 4 (right). Cross-section of 28-day IR8 endosperm showing protein bodies (p.b.) and compound starch granules (s.g.). Stain: hematoxylin.

The particulate nature of the protein bodies in a section of the 28-day kernel is evident at high magnification (Fig. 4). These particles range in size from 1 to 3 μ. More protein bodies were found along the periphery of endosperm cells than at the center. Similar protein bodies were observed in developing kernels of two other varieties. No significant change in the size of protein bodies was observed during development. In contrast, Jennings *et al.* (21) found a considerable increase in the size of protein bodies of wheat during kernel development.

In the endosperm cells of mature IR8 and other varieties, protein bodies were observed in all cases occupying the space between the compound starch granules. Some of the protein bodies were distorted, presumably because of pressure of the enlarged starch granules. The extent of protein staining increased from center to peripheral cells. Little and Dawson (22) reported an identical distribution of protein in saliva-digested cross-sections of the rice endosperm. However, they observed that the protein was mainly in the form of a membranous network, together with a few inclusions. Although the existence of rice protein in these two forms cannot be discounted, it can also be explained by the reported fragile nature of rice protein bodies (23).

The degree of protein staining was proportional to the protein content of the samples. Examples are milled rice samples of BPI-76 with 9 and 14% protein. In the 14% protein sample, bromphenol-blue staining corresponded to about 20% of the peripheral area (19) (Fig. 5, a). Protein content of the

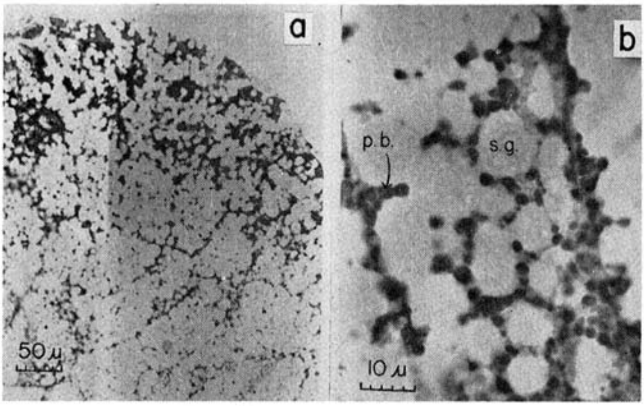


Fig. 5. Cross-sections of 14% protein BPI-76 milled rice stained with mercuric chloride-bromphenol blue, showing protein bodies (p.b.) and compound starch granules (s.g.): a) low and b) high magnification.

over-milling fraction of this layer was about 20% of dry matter (24). The particulate nature of the protein body is most evident in this high-protein sample at high magnification (Fig. 5, b). The low-protein BPI-76 kernel had as much protein body present as the IR8 kernel (Fig. 4) with the same protein content.

The main (about 80%) protein fraction of rice is glutelin (25). It is plausible to expect the protein bodies to contain the glutelin fraction. Increase in protein content of a variety is a result mainly of the increase in glutelin protein (25). Since the increase in protein content of BPI-76 kernel was accompanied by a corresponding increase in the number of protein bodies in the endosperm, glutelin must be the principal protein constituent of these storage bodies. This was recently confirmed by analysis of isolated rice protein bodies (23).

Protein bodies have previously been mentioned in histological studies of the rice endosperm by Santos (4). Raison[3] and Mitsuda *et al.* (23) similarly noted protein bodies in electron micrographs of the immature rice caryopsis. Aimi and Fujimaki (26) reported the presence of hematoxylin-staining bodies which increased in number during caryopsis development. These they termed phosphate bodies because of their positive reaction to

[3] J. K. Raison, personal communication, 1965.

molybdate reagent. The unidentified electron-dense bodies in electron micrographs of rice endosperm reported by Buttrose (27) and Kurasawa *et al.* (28) probably are proteinaceous in nature.

White-belly (abdominal white) of the caryopsis of IR8 is an interesting abnormality. The loose packing of starch granules was already present in the radially flattened cells in the ventral portion even in the 4-day kernel, and little change in starch packing of this portion of the endosperm was noted during development. Presumably the ventral portion of the endosperm and white-belly are developed during early caryopsis development. Translucence of the developing caryopsis starts from the center as seen in the 7-day kernel. In the 14-day kernel, the translucence had spread throughout the whole caryopsis, except for the abdominal or ventral portion. White-belly could be seen distinctly from the exterior of the 28-day caryopsis. Since increase in starch content during development was essentially an increase in granule size (8), white-belly is the result of the fewer starch granules synthesized in the ventral cells of the caryopsis. The air spaces may result from collapse of the dehydrating protoplasm during maturation.

In their studies of caryopsis opacity as influenced by environmental factors, Ebata and Nagato (2) reported that the ventral area is developed faster and earlier than the dorsal area during ripening. White-belly caryopses are larger than translucent ones, owing to vigorous initial growth at the ventral area, but with incomplete packing of starch. Our results are consistent with the early development of the ventral area and white-belly portion of IR8.

When the opaque portion is located at the center of the rice caryopsis, it is termed the white core. Histological examination of the white-core caryopsis of Taichung (Native) 1 revealed a lighter starch-iodine staining at the white-core or central portion in contrast with the surrounding peripheral

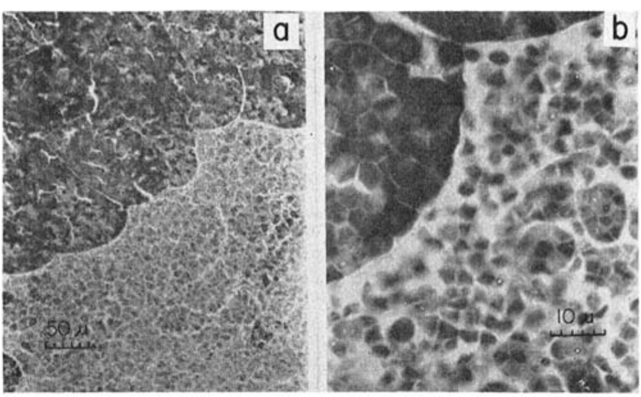


Fig. 6. Cross-sections of the center of Taichung (Native) 1 white-core milled rice stained with fast green-iodine. Lightly stained area corresponds to the white core.

cells (Fig. 6, a). On closer examination, loose arrangement of smaller starch granules also characterized the white-core portion of the endosperm in contrast with their compact arrangement and larger size in the translucent portions (19) (Fig. 6, b). Many simple granules were noted in the white-core area. A portion of the space between the starch granules stained for protein.

Still another interesting example of caryopsis opacity is crumbly rice, in which practically the whole endosperm is opaque (19). Simple and compound granules were loosely arranged throughout the endosperm cross-section (Fig. 7, a). Hematoxylin stained only a minor portion of the space between the starch granules. Hence, a major portion of this area was void space (Fig. 7, b). Comparatively greater protein-staining area was noted in cross-

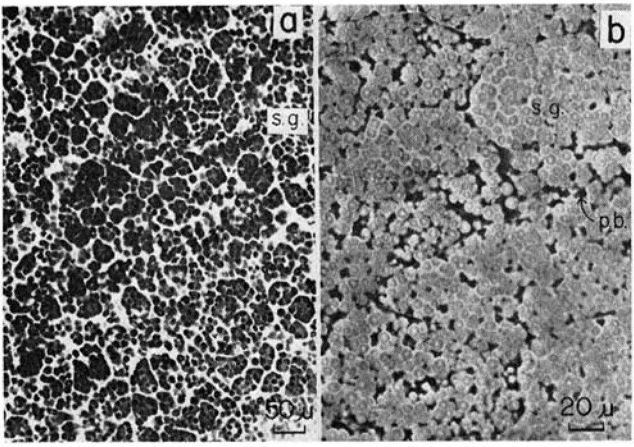


Fig. 7. Cross-section of crumbly rice endosperm with loose arrangement of starch granules (s.g.) and protein bodies (p.b.). Stain: a) fast green-iodine; b) hematoxylin.

sections of the white core and white-belly than in those of crumbly rice, even though the protein contents of the samples were similar. The loose packing of starch granules in crumbly rice is similar to that of the endosperm of *opaque-2* corn (29), but unlike *opaque-2* protein, the lysine content of its protein of 4.26% is almost the same as that of ordinary translucent brown rice (19). The crumbly rice ratio of albumin : globulin : prolamin : glutelin of 8.5:10.0:3.2:78.3 is normal except for its higher albumin and globulin contents. The brown rice contained 19.5% amylose and its starch had a gelatinization temperature of 63°–71°C. In comparison, BPI-76 and IR8 of similar protein contents have albumin-globulin:prolamin:glutelin ratios of 11.2:3.4: 85.4 (25), and 16.0:4.1:79.8 (9), respectively. The lower content of salt-soluble protein in BPI-76 may explain the reported (9,25) lower lysine content of protein in this variety than in crumbly and IR8 rices.

The opaque portions of mature rice endosperm were all characterized by loose packing of starch granules and protein-staining material in the cells involved. As with floury endosperm of corn (30), presumably the protein matrix does not completely fill the space between the loosely packed starch granules. Nagato and Kono (31) claim that white core corresponds to disorderly arrangement of center cells of the endosperm. Ueda and Ota (32) noted the presence of cracks and fissures along the dorsiventral line in the white core of rice endosperm treated with hydrochloric acid. Shibuya *et al.* (33) claim that the white core in developing caryopsis corresponds to linear and circular lacunas in sections treated with takadiastase. Such cracks may be artifacts that resulted from sample preparation, since these opaque portions are softer than the rest of the endosperm (31).

Although the protein-staining area appeared greater in the white-belly portion than in the rest of the immature IR8 caryopsis, actual chemical analysis indicated that the white-belly and the rest of the mature caryopsis have the same protein content and aminogram[4]. This suggests that the white core may be structural rather than chemical in nature and is the result of the collapse of the protoplasmic gel during kernel dehydration. Santos (4) found, by staining, mainly starch and little protein in the white-belly. Ueda and Ota (34) reported on the basis of chemical analysis that the white-core caryopsis has smaller protein and pectin contents in the center of the endosperm than in the center of translucent caryopsis of the same variety. In contrast, Shibuya *et al.* (33) found histologically that the white-core portion is rich in pectin, an anomaly between histochemical and chemical investigations. Little and Dawson (22) also reported that rice endosperm cell walls were stained with ferric-ferricyanide reagent, but its exact nature—amino acids, pectins, or other substances—was not determined. MacLeod and McCorquodale (35), in this respect, found that pectin was absent from barley endosperm cell walls, and they concluded, in certain cases, that the intercellular cementing material was proteinaceous. Further research is needed to identify this matrix conclusively.

In the waxy rice variety Malagkit Sungsong, hematoxylin and iodine staining of the endosperm of immature kernel indicated that the waxy starch granules were compound and closely arranged (Fig. 8). In this particular

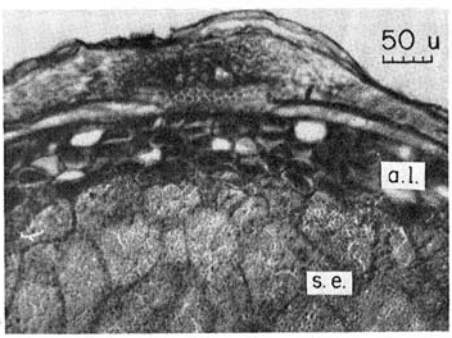


Fig. 8. Cross-section of the dorsal portion of immature endosperm of Malagkit Sungsong stained with hematoxylin: a.l., aleurone layer; s.e., starchy endosperm.

sample the aleurone cells were still nucleated, and the aleurone layer was as much as five cells thick at the dorsal portion. In addition, simple waxy starch granules were observed in the aleurone cells. The waxy characteristic of this rice starch was confirmed by its brown coloration with iodine. Buttrose

[4] M. Nazareno, unpublished data, 1966.

(36) also reported the presence of starch granules in aleurone cells of immature wheat kernel.

Presumably the nature of caryopsis opacity differs in waxy and nonwaxy rice, since the starch granules in the waxy endosperm are also closely arranged. Opacity of waxy rice endosperm may be due to the presence of micropores in the starch granules themselves, which are absent in nonwaxy rice starch granules as reported by Watabe and Okamoto (37).

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