JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparison of the Physicochemical Properties and Ultrastructure of Japonica and Indica Rice Grains

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The physicochemical properties and ultrastructures of japonica vs indica rice varieties and waxy vs nonwaxy rice varieties were compared. The viscogram values of the indica varieties were significantly higher than those of the japonica varieties. The gelatinization temperatures, breakdown, and setback were significantly lower for waxy than for nonwaxy rice varieties. Japonica rice exhibited lower hardness but higher adhesiveness than indica rice. The air space between individual starch granules was larger for waxy than for nonwaxy rice. The starch granules were compact in japonica rice, while the compound starch granules of indica rice were much smaller than those of japonica rice and were scattered widely in the endosperm. The protein bodies in japonica rice were concentrated near the cell wall, whereas those in indica rice were scattered around amyloplasts. These results suggest that the ultrastructure of rice affects the texture of the cooked product.

KEYWORDS: Rice; japonica; indica; waxy rice; gelatinization; retrogradation; ultrastructure; scanning electron microscopy; transmission electron microscopy

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for more people than any other single plant species. Rice is consumed mostly in the form of cooked whole (milled) grains. There are various types of rice: Indica rice varieties are popular worldwide, with cooked indica rice being hard but not sticky; Japanese and Koreans tend to prefer japonica cooked rice because of its moderate elasticity and stickiness, which is attributable to its lower amylose content; and glutinous rice is generally used in ready-to-eat products such as rice crackers and steamed rice cakes. This paper reports on the properties of four rice varieties: indica nonwaxy, indica waxy, japonica nonwaxy, and japonica waxy.

The physicochemical and textural properties of rice grains determine the basic food quality and palatability of the cooked product, including the overall quality, pasting properties, and texture (*1*, *2*), and these are governed by the ultrastructures of endosperm cells via the basic components of starch, protein, lipid, and fiber, as has been investigated by microscopy (*3*−*7*). This study investigated the relationship between the physicochemical properties and the ultrastructures of grain in the endosperm of various varieties of rice.

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MATERIALS AND METHODS

Materials. Four rice varieties—Hwasunchalbyeo (HSW, japonica waxy rice), Hangangchalbyeo (HGW, indica waxy rice), Ilpumbyeo (IP, japonica nonwaxy rice), and Yongjubyeo (YJ, indica nonwaxy rice)—were cultivated in the experimental field of the National Institute of Crop Science, Suwon, in 2002. The rice samples were milled using a MC-90A polisher (Toyo, Japan) to a milling yield of 90%, and various physicochemical properties of both raw and cooked milled rice grains were analyzed. For the ultrastructural study, grains of rice were harvested 20 days after flowering (DAF), since rice starch endosperm has been reported to be mature at this time, with no noticeable differences in the ultrastructures of endosperm samples from those harvested at later times (*8*, *9*).

Starch Isolation. Starch was isolated using the method described by Hoover and Sosulski (*10*), which involved repeated steeping of rice flour in 0.2% aqueous NaOH solution. The isolated starch was dried at room temperature, ground into powder, and passed through a 100 mesh sieve.

Proximate Compositions and Amylose Content. The moisture, ash, crude protein, and crude fat contents were quantified using standard AOAC methods (*11*). The amylose content was determined using the simplified assay method of Juliano (*12*).

Rheological Properties. The pasting properties of rice flours were measured by AACC-approved method 61-02 (*13*) using a rapid viscoanalyzer (RVA; Newport Scientific, Warriewood, NSW, Australia).

Thermal Properties. The thermal properties of rice starches were analyzed using a differential scanning calorimeter (model SSC/5200, Seiko, Japan). Rice starch samples (5 mg) were weighed in aluminum

10.1021/jf060221+ CCC: $33.50
Published on Web 05/23/2006

Table 1. Varietal Differences in the Proximate Composition of Milled Rice Unit: %, w/w

variety	moisture	ash	protein	fat	amylose
			waxy		
HSW	9.94 ± 0.16[b]	0.51 ± 0.05	5.68 ± 0.05 b	1.10 ± 0.00 b	
HGW	10.47 ± 0.17	0.61 ± 0.03	6.59 ± 0.05 a	1.32 ± 0.00 a	
D[a]	−0.53	−0.10	−0.91***[c]	−0.22**	
			nonwaxy		
IP	9.85 ± 0.10	0.37 ± 0.01	6.66 ± 0.16 b	0.44 ± 0.00 b	18.63 ± 0.67
YJ	9.99 ± 0.09	0.48 ± 0.07	7.32 ± 0.07 a	0.55 ± 0.01 a	17.74 ± 1.13
D	−0.14	−0.11	−0.66**	−0.11**	0.89

[a] D, difference between japonica and indica varieties. [b] Mean ± SD ($n = 3$). [c] **, ***, significant at 0.01 and 0.001 levels, respectively. Different letters within the same column indicate significant differences.

pans, mixed with distilled water (10 μL), sealed, and allowed to stand for 1 h at room temperature. A heating rate of 5 °C/min was applied from 30 to 160 °C. After they were cooled, the samples were stored in a refrigerator at 4 °C for 7 days. Retrogradation was measured by reheating the sample pans containing the starch samples at a rate of 10 °C/min from 30 to 160 °C. The percentage retrogradation was calculated as the ratio of the enthalpy of retrogradation to the enthalpy of gelatinization (*14*).

Texture Analysis of Cooked Rice. Water was added to milled rice samples (30 g) in a stainless steel cup (60 mm diameter and 70 mm deep) to give a rice:water weight ratio of 1:1.25. After the cups were soaked for 20 min at room temperature, the stainless steel cup containing water and soaked rice grains was placed in an electronic rice cooker (LG, Korea). The rice in the cup was then steamed for 30 min, followed by a 10 min holding period after which texture profile analysis (TPA) was performed on the rice in the stainless steel cup using a texture analyzer (TA-XT2, Stable Micro System, United Kingdom) at room temperature. A two-cycle compression, force-vs-time program was used with a test speed of 2 mm/s and a rate of 80% strain using a cylinder plunger with a diameter of 20 mm.

The parameters derived from the test curves were hardness, adhesiveness, cohesiveness, and springiness. Chewiness was quantified by multiplying the gumminess and the springiness. The parameters of the TPA curve were determined as described by Bourne (*15*) and defined by Munoz (*16*).

Scanning Electron Microscopy. Individual grains were fractured in the midregion using a razor blade by applying slight pressure to the top of the grain, while taking care to ensure that there was no physical contact between the razor blade and the fractured surfaces of the internal endosperm tissues. Fractured rice grains, with the fractured surface upward, were immediately mounted on a specimen stub and sputter coated with gold before being viewed with a scanning electron microscope (JSM 5401 LV, JEOL) operating at 30 kV.

Transmission Electron Microscopy. Grains of the four rice varieties (harvested at 20 DAF) were sliced with a razor blade in the midregion, with each slice further cut into several tissue blocks of approximately 1−2 mm^3, each of which included the peripheral and central regions of the endosperm. The 20 DAF grains were already hardened, which made it difficult to make clean cuts. However, we made every effort to ensure that each tissue block included both regions. The tissue blocks were then immediately fixed by placing them in a modified Karnovsky's fixative (*17*) containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.2, and were left therein for 24 h at room temperature. After two 10 min washes with the same buffer solution, the blocks were postfixed in 1% osmium tetroxide (buffered in 0.05 M cacodylate buffer) for 2 h at room temperature. The blocks were stained en bloc in 0.5% aqueous uranyl acetate overnight at 4 °C, dehydrated through an ethanol series from 30 to 100%, and embedded in Spurr's low-viscosity embedding medium (*18*). Several of the sections were thin and of poor quality, probably due to the nature of the spreading endosperm tissue, which consists primarily of starch. Most of these thin sections had numerous folds and torn areas, as has been noted by other investigators (*19*). The thicker sections were of a higher quality and were cut with a diamond knife and stained with 2% aqueous uranyl acetate for 5 min followed by lead citrate for 2 min. Grids were viewed with a transmission electron microscope (LEO 906, Zeiss) operating at 80 kV. The endosperm composition including starch and protein bodies (PBs) was identified based on dying density and rice immunohistology (*8*, *19*).

Table 2. Mean Grain Sizes and Shapes of Rice Samples

variety	length (mm)	width (mm)	thickness (mm)	length/width
		waxy		
HSW	4.93 b	2.77 a	1.88 a	1.78 b
HGW	5.93 a	2.47 b	1.76 b	2.39 a
D[a]	−1.00***[b]	0.30***	0.12***	−0.61***
		nonwaxy		
IP	4.58 b	2.90 a	2.00a	1.58 b
YJ	5.35 a	2.53 b	1.76 b	2.11 a
D	−0.77***	0.37***	0.24***	−0.53***

[a] D, difference between japonica and indica varieties. [b] ***, significant at the 0.001 level. Different letters within the same column indicate significant differences.

Data Analysis. Physicochemical analyses were performed in triplicate, and the texture analysis of each variety of cooked rice was repeated on 20 replicated samples. The data are presented as mean ± standard deviation (SD) values. Statistical data were assessed by analysis of variance using commercial software (SAS v. 8.0, SAS Institute, Cary, NC), with differences considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Proximate Composition and Grain Size. The amounts of protein and fat were higher in the indica rice varieties (YJ and HGW) than in the japonica rice varieties (HSW and IP) (**Table 1**), and there was more ash in the waxy rice varieties (HSW and HGW) than in the nonwaxy rice varieties (IP and YJ). The fat content in HSW and HGW (1.10−1.32%) was twice that in IP and YJ (0.44−0.55%). The amylose content, which is related to the stickiness of cooked rice, was similar in IP (18.63%) and YJ (17.74%), classifying these as low-amylose varieties based on the cutoff of the International Rice Research Institute (IRRI, Manila, Philippines) (*20*). The amylose content of the waxy rice varieties was negligible.

The length of milled rice grains decreased in the order HGW > YJ > HSW > IP (**Table 2**), with the width and thickness increasing in the same order. According to the ratio of grain length to width, japonica rice varieties were classified as short grains and indica rice varieties were classified as intermediate-to-long grains (**Figure 1**); this figure also shows that the transparency of waxy rice endosperms was lower than that of the nonwaxy rice endosperms.

Pasting Properties. Table 3 lists the pasting properties of the rice flours, as measured by the RVA. The viscogram values of the indica varieties were significantly higher than those of the japonica varieties, with the peak viscosity varying the most

Table 3. Varietal Differences in Pasting Properties of Rice Flours Measured by an RVA

variety	initial pasting temp. (°C)	viscosity (RVU)[a] peak	hot-paste	final	breakdown[b]	setback[c]
			waxy			
HSW	67.67 ± 0.055[e] a	107.50 ± 34.21	57.33 ± 3.05 b	76.33 ± 3.78 b	51.5 ± 4.94 b	−32.50 ± 3.54 a
HGW	64.10 ± 0.70 b	190.00 ± 2.82	85.00 ± 1.41 a	116.50 ± 3.53 a	105.00 ± 4.24 a	−73.50 ± 6.36 b
D[d]	3.57**[f]	−82.50	−27.67**	−40.17**	−53.50***	41.00*
			nonwaxy			
IP	69.15 ± 0.07 b	205.00 ± 18.38 b	86.00 ± 4.24 b	172.00 ± 5.65 b	119.00 ± 22.62	−33.00 ± 1.04
YJ	72.50 ± 0.14 a	311.50 ± 9.19 a	123.50 ± 6.36 a	207.00 ± 9.89 a	188.00 ± 2.82	−104.50 ± 0.71
D	−3.35**	−106.50*	−37.50*	−35.00*	−69.00	71.00

[a] RVA units. [b] Peak viscosity minus hot viscosity. [c] Final viscosity minus peak viscosity. [d] D, difference between japonica and indica varieties. [e] Mean ± SD ($n = 3$). [f] *, **, and ***, significant at 0.05, 0.01, and 0.001 levels, respectively. Different letters within the same column indicate significant differences.

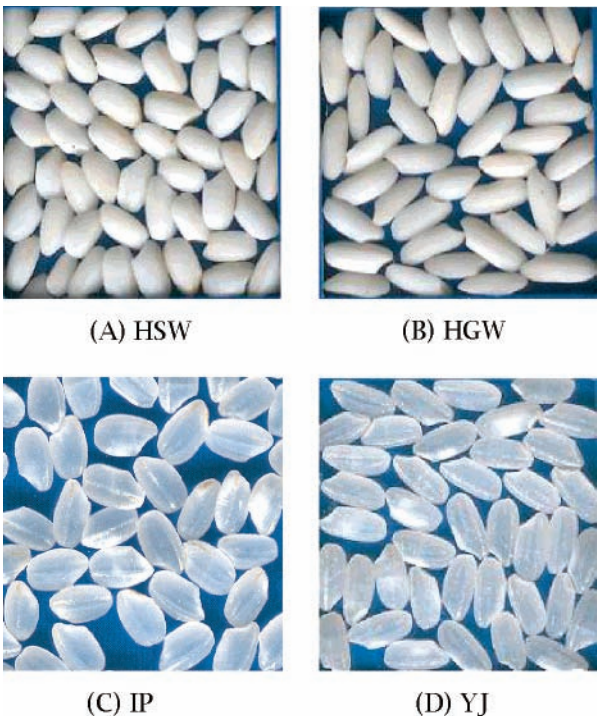

Figure 1. Photographs of milled rice grains. The opaqueness of waxy rice endosperms (**A**, **B**) contrasts with the transparency of nonwaxy rice endosperms (**C**, **D**). Japonica rice varieties (**A**, **C**) and indica rice varieties (**B**, **D**) were classified as short and intermediate-to-long grains, respectively.

(by about 90−100 RVA units) for the waxy and nonwaxy rice varieties. The waxy rice varieties exhibited significantly lower pasting temperatures and viscosities—especially at breakdown and setback—as compared with nonwaxy rice varieties. The pasting properties of the waxy rice varieties are similar to those reported by Vandeputte et al. (*21*).

Thermal Properties. The thermal properties of rice starches in terms of gelatinization and retrogradation are listed in **Table 4**. The transition temperatures and enthalpies for gelatinization were slightly higher for the waxy rice varieties than for the nonwaxy rice varieties, which may be attributable to differences in amylose content, starch structure, and the content of amylose−lipid complex. Nakazawa et al. (*22*) reported that the gelatinization transition temperature was higher for waxy than for nonwaxy rice starches, due to the former being more crystalline and consequently more resistant to gelatinization. Because amylopectin plays a major role in starch granule crystallinity, the presence of amylose lowers the melting point of crystalline regions and the energy at which gelatinization begins, since more energy is needed to initiate melting in the absence of amylose-rich amorphous regions. The differences in transition temperatures between rice starches may also be attributable to differences in the chain length distributions of the amylopectin (*23*). Moreover, these results are in line with observations of Abdel-Aal et al. (*24*), who found that in comparison with nonwaxy wheat, waxy wheat starch exhibited higher gelatinization temperatures, a greater degree of crystallization, and an absence of an amylose−lipid complex.

The endothermic peaks after storing the gelatinized rice starches for 7 days at 4 °C appeared between 40.24 and 49.44 °C. The percentage retrogradation increased in the order HSW

Table 4. Varietal Differences in Thermal Properties of Rice Starches for Gelatinization and Retrogradation as Measured by a Differential Scanning Calorimeter

	gelatinization			retrogradation			
variety	T_{onset} (°C)	T_{peak} (°C)	ΔH (mJ/mg)	T_{onset} (°C)	T_{peak} (°C)	ΔH (mJ/mg)	retrogradation ratio (%)[a]
				waxy			
HSW	62.77	68.07	12.50 a	40.48	42.96	0.13 b	1.04 b
HGW	62.90	68.03	11.66 b	49.44	56.19	0.50 a	4.29 a
D[b]	−0.13	0.04	0.84**[c]	−8.96	−13.23	−0.37***	−3.25***
				nonwaxy			
IP	58.02	65.27	10.76 a	42.25	51.63	1.31 b	12.21 b
YJ	61.65	66.61	10.50 b	40.24	53.67	1.62 a	15.44 a
D	−3.63	−1.34	0.26*	2.01	−2.04	−0.31**	−3.23**

[a] Ratio, enthalpy of retrogradation/enthalpy of gelatinization. [b] D, difference between japonica and indica varieties. [c] *, **, and ***, significant at 0.05, 0.01, and 0.001 levels, respectively. Different letters within the same column indicate significant differences.

Table 5. Varietal Differences in Texture Properties of Cooked Rice Measured by TPA

variety	hardness (g)	adhesiveness (gcm)	springiness (cm)	chewiness (gcm)	balance (hardness/ adhesiveness)
			waxy		
HSW	828.40 b	356.66 a	0.81	0.20	0.43 a
HGW	908.20 a	308.78 b	0.78	0.19	0.34 b
D[a]	−79.80**[b]	47.88**	0.03	0.01	0.09*
			nonwaxy		
IP	949.90	158.85	0.76	0.17	0.17
YJ	955.30	158.21	0.69	0.18	0.17
D	−5.40	0.64	0.07	−0.01	0.00

[a] D, difference between japonica and indica varieties. [b] * and **, significant at 0.05 and 0.01 levels, respectively. Different letters within the same column indicate significant differences.

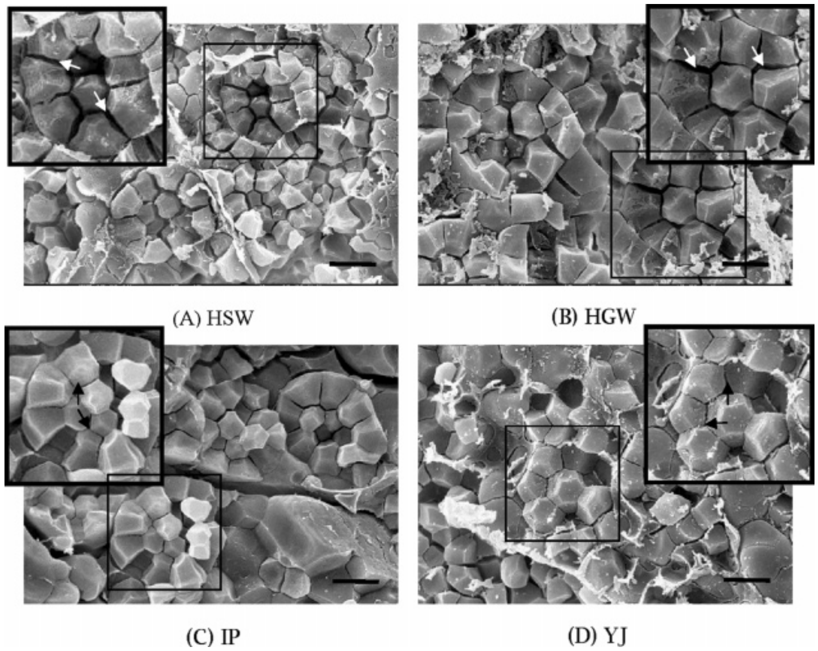

Figure 2. Ultrastructure of rice endosperms at 20 DAF by scanning electron microscopy at a magnification of 10000×. The air space between individual starch granules within compound starch granules was larger in waxy rice varieties (**A**, **B**) than in nonwaxy rice varieties (**C**, **D**) (scale bar = 5 μm). The inserts show the areas of the square boxes in the main images at higher magnification, revealing the air spaces between starch granules.

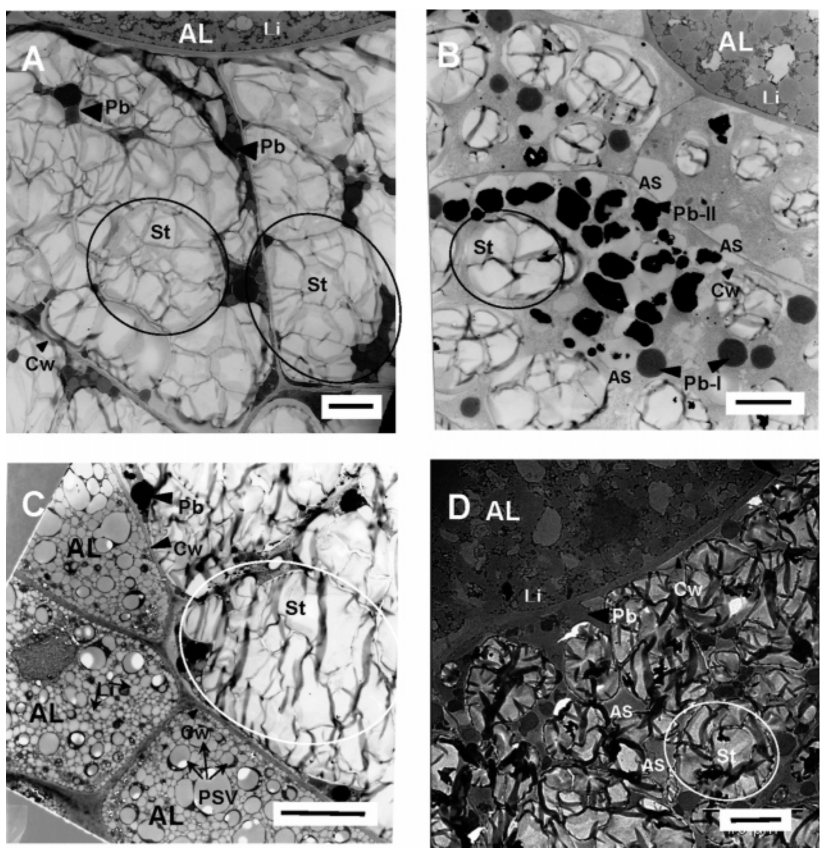

Figure 3. Low-magnification transmission electron microscopy images demonstrating the aleurone cell layer and subaleurone starch endosperm cells from HSW (**A**), HGW (**B**), IP (**C**), and YJ (**D**) (scale bar = 5 μm). Larger amyloplasts (circle) packed with fluffy starch granules (St) are more prevalent in japonica rice varieties (**A**, **C**) than in indica rice varieties (**B**, **D**). AL, aleurone cells; Li, lipid bodies; PSV, protein storage vacuoles; St, starch granules; AS, air space; and Cw, cell wall.

< HGW < IP < YJ. The differences in retrogradation enthalpy may be attributable to differences in amylose:amylopectin ratios and the chain lengths of amylopectin. Indica rice amylopectin has longer chains than japonica amylopectin (*25*), producing a higher staled melting endotherm in the former.

Texture of Cooked Rice. Table 5 lists the following texture properties of cooked rice as measured using a texture analyzer: hardness, adhesiveness, springiness, and chewiness. Waxy rice varieties exhibited a lower hardness but significantly higher adhesiveness and balance index (adhesiveness/hardness). The

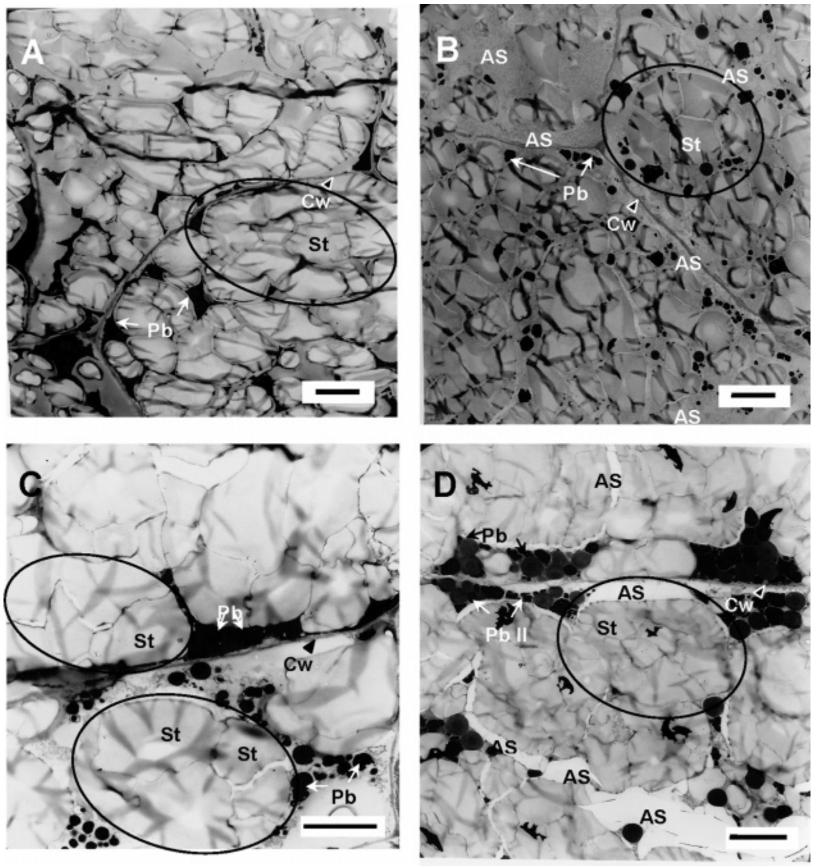

Figure 4. Higher magnification transmission electron microscopy images of two subaleurone starch endosperm cells from HSW (**A**), HGW (**B**), IP (**C**), and YJ (**D**) (scale bar = 5 μm). In japonica rice varieties (**A**, **C**), the starch granules were so compact that voids were virtually absent in the endosperm cells. Circle, amyloplast; St, starch granules; AS, air space; and Cw, cell wall.

texture of waxy rice is related not only to the structure of amylopectin but also to the amylose and protein contents and the gelatinization temperature (*26*). Japonica rice varieties exhibited lower hardness but higher adhesiveness and springiness values than indica rice varieties, in both the waxy and the nonwaxy groups.

Scanning Electron Microscopy Results. Figure 2 shows the transversely fractured surfaces of the midregion of rice grains at 20 DAF. The endosperms of the four rice varieties had similar morphology when viewed by scanning electron microscopy. The endosperm region comprised masses of starch granules and cell wall materials, and the starch granules were polygonal and clustered into amyloplasts or compound starch granules. Compound starch granules were either unsplit or partially split. The average diameters of starch granules of indica rice varieties (HGW and YJ) were larger than those of japonica rice varieties (**Figure 2**), which differs from the report of Hoshikawa (*27*). The air space between individual starch granules within compound starch granules was larger for waxy than for nonwaxy rice varieties, which may have been responsible for the relative opaqueness of the waxy rice endosperm. It has been demonstrated (*28*) that the opaqueness of waxy rice is due to the loose packing of starch granules, with micropores and indentations being present on the outer surfaces of single starch granules and compound starch granules, respectively.

Transmission Electron Microscopy Results. An aleurone layer and starchy endosperms of rice grains harvested at 20 DAF were evident in transmission electron microscopy images (**Figures 3** and **4**). The aleurone cells were similar in the four rice varieties, being composed of aleurone PBs and lipid bodies (**Figure 3**). The density of subaleurone starchy endosperm of compound starch granules differed among the rice varieties. In japonica rice varieties, the starch granules were so compact that voids in the endosperm cells were almost absent (**Figure 3**), whereas the compound starch granules of indica rice (which were much smaller than those of japonica) were scattered widely in endosperm cells (**Figure 3**). Two types of PBs—spherical prolamine PBs (PB-I) and segmented glutelin PBs (PB-II)—were observed among the starch endosperm cells. PBs in japonica rice varieties were concentrated near the cell wall, whereas those in indica rice varieties were scattered throughout the cell (around the starch granules). The distribution of PBs in indica rice appears to prevent water from entering the starch granules and being absorbed, resulting in incomplete gelatinization and harder cooked rice.

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Received for review January 25, 2006. Revised manuscript received April 14, 2006. Accepted April 23, 2006.

JF060221+