Endosperm Structure of White-Belly and White-Core Rice Grains Shown by Scanning Electron Microscopy

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Abstract: White-belly and white-core are the major two types of grain chalkiness in japonica rice. This study aims to compare the morphological features of white-belly and white-core using a scanning electron microscope (SEM). A japonica rice cultivar Wuyujing3 and its mutants were used as materials. Nearly 1000 SEM images were observed, and 12 representative photos were selected. SEM images showed contrasting differences between white-belly and white-core in endosperm microstructure including the shape of endosperm cell, the size distribution of starch granules, and the amount of protein bodies. White-belly and white-core also varied markedly in morphological features of the cracked compound starch granules. Our findings should help to advance our understanding of the multi-faceted nature of grain chalkiness from the perspective of starch and protein accumulation, and should be of value for future work on rice grain chalkiness.

Key words: Grain chalkiness, SEM, White-belly rice grain, White-core rice grain.

Grain appearance is an important criterion in determining the quality of rice. Most rice consumers essentially prefer uniform and translucent grains. Chalkiness is an opaque area in the rice grain. It reduces the resistance of grains to forces exerted during the milling process, decreasing the amount of edible rice. It is also an undesirable characteristic because it is inferior in visual appearance, adversely affecting consumer acceptability and thereby lowering the overall market value (Singh et al., 2003). High temperatures during grain filling facilitate the formation of chalky grains. Current climate models project that mean global temperature will continue to increase as a consequence of the greenhouse gas effect, and the grain chalkiness caused by high temperature stress should become a significant threat to rice agriculture in the future (Fitzgerald et al., 2009; Lanning et al., 2011; Li et al., 2011).

According to the position of the opaque part, chalky rice is categorized into white-belly rice, white-core rice, white-base rice, and white-back rice. Among them, white-belly and white-core rice are the major two types for japonica rice. White-core rice has an opaque part in the centre of the endosperm, while white-belly rice has chalkiness in the ventral part. It is assumed that white-core occurs when environmental conditions are unfavorable or poor translocation of assimilates from leaves and stems at early and middle stages of grain filling (Taira, 1995). By contrast, white-belly is speculated to occur at the late stage due to detrimental environments or insufficient utilization of reserve substances (Taira, 1995). In addition, the differences in the location of chalkiness can be used to explain the different response of white-belly and white-core to high-temperature stress (Tsukaguchi and Iida, 2008) and alteration of source-sink ratio (Cheng et al., 2007). Thus, it is necessary to study white-belly and white-core separately when exploring the genetic or physiological causes of chalkiness.

There have been numerous reports on the endosperm structure of chalky grains examined using a scanning electron microscope (SEM). A common feature of the opaque tissues, the disorganized starch granules, was noted (Del Rosario et al., 1968; Tashiro and Ebata, 1975; Lisle et al., 2000; Fitzgerald et al., 2009; Ishimaru et al., 2009; Zhou et al., 2009). We compared morphological traits between white-belly and white-core tissues, and found that most starch granules in white-belly tissues were surrounded by globular protein bodies, with many air spaces between them. On the other hand, starch granules in white-core tissues were easily broken into single granules, while no protein bodies were visible (Qiao et al., 2011). However, little information is available regarding the typical difference between white-belly and white-core, the role of proteins in chalkiness formation, and the explanation for the fragility of starch granules in white-core tissues.

We further compared the morphology of white-belly...
and white-core endosperm using a japonica rice cultivar and its mutants. About 1000 SEM images of chalky grains were obtained, enabling us to give a detailed description of the morphological features of chalkiness. Here typical photos are selected, and the objectives are (1) to define typical morphology of white-belly and white-core tissues, and (2) to exhibit representative features of cracked starch granules of white-belly and white-core. The results obtained may help to clarify the physiological foundation of white-belly and white-core and be of value for future work on rice grain chalkiness.

Materials and Methods

Wuyujing3 and its mutants were used in this study. Wuyujing3 is a benchmark cultivar of China with high eating quality. However, it has weak points such as relatively lower yield potential, susceptibility to rice stripe virus, and high chalky grain rate, usually being above 30%. In 2007, 2000 g seeds of Wuyujing3 were presoaked for 6 hours in distilled water and then treated with 0.5% ethyl methane sulfonate (EMS) for 16 hours at room temperature of about 25°C. After the treatment, the seeds were thoroughly washed. Before sowing, seeds were soaked for 24 hours and then drained 24 hours. The mother plant (M1) was grown in the field and mixed harvested.

In 2009, seeds of the M1 plant were sown in the field as M2 generation. M2 plants that differed from the M1 plants were marked, and panicles on the main tillers were harvested individually. Seeds of the M2 panicle were sown in separate rows in nursery beds. The seedlings were transplanted in separate rows as panicle-row progeny.

M1 seeds were selected for chalkiness after harvest of M2
plants in 2009. Three mutants were identified, with one having a high rate of white-belly grains whereas the other two having a high percent of white-core grains. In 2010, the three mutants were grown in the field, without visible variations among M3 plants in the agronomical traits of plant height, panicle type, and growth duration. Importantly, the occurrence of chalkiness in M4 seeds of each mutant was nearly the same as M3.

M4 plants were grown in field conditions, demonstrating stable agronomical traits. At maturity, about 30 panicles with similar maturity were harvested. The samples were naturally dried and dehulled. Grains with a large area of white-belly and white-core were chosen, and were completely dried under low pressure. The dried grains were transversely cut with a razor blade, producing a fracture rather than a clear cut. The fracture was sputter-coated with gold in vacuum and observed using a SEM (Hitachi S-3000N) at an accelerating voltage of 15 kV.

Results

1. Typical Morphology of White-Belly and White-Core Tissues

We obtained about 1000 photos of the endosperm structure of white-belly, white-core, and their translucent counterparts. However, most of them are of poor quality, because the specimen was randomly cut with a razor blade and produced a fractured structure. The majority of starch granules are easily broken by mechanical stress, particularly in the opaque tissues in the ventral and central part. Thus, it is difficult to obtain a high-quality photo showing an intact endosperm cell in which all starch granules are intact. Among these photos, high-quality photos showing a clearly different structure of white-belly and white-core tissues are shown in this paper.

Fig. 1 shows the structural differences between white-belly and white-core tissues. Starch granules and protein bodies are tightly packed in the translucent ventral part.
(Fig. 1A) and central part (Fig. 1D). In addition, the SEM photo shows clearly the more slender shape of the endosperm cell in the translucent central part than that in the ventral part. For the chalky tissues, white-belly and white-core are different in the shape of endosperm cells, the amount of protein bodies, and the size distribution of starch granules. Endosperm cells in the ventral part (white-belly tissue) are rectangular in shape (Fig. 1B); while those in the central part (white-core) are relatively slender (Fig. 1E). In white-belly tissues, starch granules are intact and surrounded by globular protein bodies, with obvious intergranular air spaces (Figs. 1B and 1C). This result agrees with that reported by Tashiro and Ebata (1975). Conversely, fewer protein bodies are observed in white-core by SEM, as reported in our previous work (Qiao et al., 2011). For the size distribution of starch granules, white-core tissues have a larger amount of small starch granules than the white-belly tissues (Figs. 1E and 1F). The diameter of starch granules in Fig 1B and 1E was measured at a high magnification. The results showed that the average diameter was 11.55 μm for white-belly, with a coefficient of variation (CV) being 17.8%; while the average was 7.29 μm for white-core, with a larger CV of 43.6%.

2. Typical Features of Cracked Starch Granules of White-Belly and White-Core

White-belly and white-core differ in the fragility of compound starch granules, as shown in Fig. 2. Compound granules of white-belly are hard to break under mechanical stresses such as the shear cut by a razor during sample preparation for SEM. By contrast, those of the white-core easily fall apart into single granules, as also reported by Lisle et al. (2000). This is partially associated with the strong protein-starch adhesion. Fig. 2A shows that the starch granules are covered with a thin protein matrix in white-belly tissue, as was reported by Tashiro and Ebata (1975). Embedded in the protein matrix are protein bodies composed of a protein. The relatively harder endosperm of white-belly may be a result of strong adhesion between protein and starch. Conversely, few if any visible protein bodies are detected in the white-core tissues, rendering them the fragility when treated with a razor.

In addition, SEM images note prominent differences in the morphological traits of cracked compound starch granules between white-belly and white-core. As shown in Figs. 2C and 2E, the single granules for white-belly are regular and generally polygonal in shape, while most of the single granules of white-core are irregular in shape with sharp edges. This may be explained on the base of the size of starch granules. Compound starch granules of white-belly are larger and made up of more single granules (Fig. 2B). On the contrary, most of the compound starch granules in the white-core are relatively small and are composed of fewer single granules (Fig. 2E). The schema of the broken feature of large and small compound granules is illustrated in Fig. 3.

Discussion

1. Role of Protein Bodies in Chalkiness Occurrence

Starch is the major component in rice grain, playing a crucial role in determining rice textual and sensory properties. So far, the majority of studies with respect to grain chalkiness have been focused on starch, relating its accumulation or degradation to the formation of chalky tissue (Yamakawa et al., 2007; Fitzgerald et al., 2009). By contrast, the role of the other main component, the proteins, had been underestimated. In rice, proteins account for about 8% of the endosperm’s weight, filling the space between starch granules. For white-belly, the present findings suggest that the insufficient accumulation of protein bodies that do not completely fill the air spaces between starch granules may be an explanation for white-belly occurrence, as was reported by Del Rosario et al. (1968) and Qiao et al. (2011). For white-core, lack of proteins is evidenced by the susceptibility of compound starch granules to cracking because of weak adhesion between them (Figs. 2D, 2E and 2F). Thus, the shortage of protein bodies is responsible for the occurrence of white-core. Generally, the crucial role of proteins in chalkiness formation appears warranted. We therefore suggest that more attention be paid to proteins as well as starch in...
studies on the physiological cause of chalky grain.

In addition, proteins in the rice grain show an uneven distribution, being more concentrated in the aleurone layer, the embryo, and the subaleurone layer of the endosperm as compared to the inner starchy endosperm (Ellis et al., 1987). In this study, we did not compare the protein concentration in the chalky tissues, either for the ventral part or for the central part. Thus more work is needed to clarify whether the incomplete accumulation of protein bodies is one of the major causes of chalky tissue formation. It is necessary to compare the chemical composition, mainly starch fine structure and protein concentration and composition, of the opaque tissues and their counterpart translucent tissues.

2. Chemical Composition of the Chalky Grains

Research has shown that chalky grains differ from translucent kernels with respect to physicochemical, morphological, thermal, and textural properties (Kim et al., 2000; Singh et al., 2003). As observed in the present study, there are obvious inter-granule spaces in the chalky part of both white-belly and white-core tissues, the result of disorganized packing of starch granules. This finding indicates that the concentration of starch is lower per square millimeter in chalky than in translucent centers. This can explain why chalky grains contained less amylose compared with translucent grains (Lisle et al., 2000; Singh et al., 2003). However, the error inherent in current methods of starch analysis could mask small differences in starch concentration. In the amyllopectin fine structure, Lisle et al. (2000) found no significant differences between chalky and translucent grains in the amount of high or low molecular weight glucans in the amyllopectin fraction. By contrast, Patindol and Wang (2003) reported that chalky grains had shorter average chain length of amyllopectin, higher percentage of short amyllopectin branch chains, and lower percentage of long branch chains. The disagreement may be partially associated with the subtle difference in amyllopectin fine structure as well as the analytical method. Nonetheless, more sensitive techniques should be used to ascertain whether chalky and translucent grains indeed differ in starch and protein compositions, the result of which will help to clarify the mechanism responsible for chalkiness formation and thus provide directions in rice breeding, genetic modification, and crop management.

3. Understanding Chalkiness from the Perspective of Carbon and Nitrogen Metabolism

Although China produces and consumes the largest volume of japonica rice, and great progress in genetic improvement of rice quality has been achieved, high chalky grain rate still exists with the new released rice cultivars, especially in the warmer southern areas (Yang et al., 2013). Eliminating chalkiness from rice has been the most important challenge for plant breeders. Grain chalkiness is a complex trait as influenced by genetic control as well as environments like high temperature. Although the heritability of chalkiness has been demonstrated by QTL mapping, genes associated with it have not been identified or cloned (Fitzgerald et al., 2009; Qin et al., 2009; Zhou et al., 2009), and the molecular mechanisms underlying its formation are poorly understood (Liu et al., 2010). The slow progress in the study of chalkiness partially reflects the complexity of mechanism underlying chalkiness occurrence. Results of comparison of white-belly and white-core tissues highlight the significance of proteins for chalkiness occurrence. In general, both starch and proteins are important for the formation of chalkiness, and therefore should be paid equal attention in future studies.

In rice grain, starch and protein are the end-products of carbon (C) and nitrogen (N) metabolism. C and N interact at numerous points in plant metabolism. Metabolic intermediates of C metabolism including glycolysis and citric acid cycle provide the carbon skeleton used for amino acid (N) biosynthesis (Pessarakli, 2001). This interdependence in C and N metabolism creates problems when one is attempting to describe an independent role for either C or N in chalkiness formation. In addition, these interactions not only occur in the sink organs of grains, but exist also for the source organs like leaves, complicating any studies on chalkiness (Pessarakli, 2001).

From analysis of the sucrose level in inferior and superior spikelets, Lisle et al. (2000) suggested that chalkiness was not due to insufficient vascular supply of metabolites like sucrose and amino acids, but was more likely related to metabolic events within the developing grain that utilize carbon in sucrose for starch deposition. Patindol and Wang (2003) reported that chalky grains had shorter average chain length of amyllopectin, which indicated that the formation of chalkiness might be associated with interruption in the step of chain length elongation. These two studies offer novel perspective for understanding the physiological reasons for chalkiness. However, there is still limited information particularly on protein synthesis or nitrogen metabolism. Therefore, we argue that it is imperative to advance our understanding of chalkiness by relating accumulation of starch and proteins in endosperm to metabolism of C and N in both sink and source organs.

Conclusions

White-belly and white-core are the two major types of grain chalkiness in japonica rice. Little information is available regarding the typical difference in endosperm structure between white-belly and white-core. Using a SEM, we compared the morphology of endosperm between
white-belly and white-core grains of a japonica rice cultivar (Wuyujing3) and its mutants. About 1000 SEM images of chalky grains were obtained, among them 12 representative photos of white-belly and white-core tissues were selected. SEM images showed contrasting differences between white-belly and white-core in endosperm microstructure including the shape of endosperm cell, the size distribution of starch granules, and the amount of protein bodies. White-belly and white-core also varied markedly in morphological features of the cracked compound starch granules. These findings should help to advance our understanding of the multi-faceted nature of grain chalkiness from the perspective of starch and protein accumulation.

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