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Analysis on the Occurrence of Chalky Rice Grain Taking into Consideration All of the Grains within a Panicle

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The objective of this study was to clarify the effect of temperature during initial ripening stage and assimilation in chalky rice grain. Japonica rice cultivars: Fusaotome, Fusakogane, Akitakomachi, Haenuki, Sasanishiki, and Koshihikari, showed different percentages in the occurrence of chalky rice grain under high temperatures during the initial ripening stage. The above Japonica rice cultivars were used to clarify the order of all grains within a panicle, and investigate rice grain weight and rice quality at different spikelet positions. Significant negative correlation between the flowering date and rice grain weight in all cultivars suggests that rice grain weight is affected by the length of the ripening period. There was also a significant positive correlation between the percentage of chalky rice grain and the days from heading to flowering in Haenuki and Sasanishiki cultivars. However, a significant negative correlation was found between the percentage of chalky rice grain and days from heading to flowering in Fusaotome, Fusakogane and Akitakomachi. In addition, the daily average temperature during initial ripening stage in Fusaotome, Fusakogane and Akitakomachi was higher than the other cultivars. These results suggest that the days from heading to flowering within a panicle and high temperature at initial ripening period cause the occurrence of chalky rice grain.

Key words: chalky rice, all grains in a panicle, days from heading to flowering, high temperature

1. Introduction

Rice is the world's single most important food crop and the primary source of calories for approximately half of the human population (Khush, 1997). Especially, rice quality is one of the most important characteristics, as it exerts a large effect on the market value and consumer acceptance. Recently, frequent occurrence of chalky rice grain has been reported nationally in Japan. Chalky rice grain occurs when the rice grain has a white portion within the rice grain. Because Japanese people do not prefer chalky rice, the frequent occurrence of chalky rice grain pushes the rice price low, which is one recent problem that Japanese rice-producing farmers face.

Microscopic observation of the chalky part of high temperature-ripened grains revealed that loosely packed starch granules create air spaces between themselves to randomly reflect light (Tashiro and Wardlaw, 1991a; Zakaria *et al*., 2002). In addition, it has been reported that high temperature during the milky stage of grain filling has the greatest influence on rice grain chalkiness (Nagato and Ebata, 1965; Tashiro and Wardlaw, 1991a). The panicle is the most sensitive organ to high temperature (Sato and Inaba, 1973; Morita *et al*., 2004). On the other hand, poor starch accumulation in the endosperm of chalky rice implies that chalky rice grain is caused by a deficit in assimilation products due to high temperature conditions. Generally, assimilation products produced by photosynthesis in the leaf move to the grain through the transport system. However a high temperature increases respiration rate, which then decreases the amount of assimilation products for one spikelet (Vong and Murata, 1977; Hirai *et al*., 2003). These results suggest the reduction in assimilation from vegetative organs due to an increase in respiration rate under high temperature conditions is not the only factor causing chalky rice grain.

Nevertheless, many experiments indirectly suggest a

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relationship between deficit in assimilation products, high temperature during ripening period and occurrence of chalky rice grain. However, the combined effects of supply of assimilation products and high temperatures on the occurrence of chalky rice grain have not been well studied. The objective of this study was to clarify the combined effects of assimilate supply and high temperature on the occurrence of chalky rice grain by consideration of all of grains within a panicle. Anthesis usually starts at the top of the panicle. Cultivars with large panicles show the high percentage of unfilled spikelets on the lower third of the panicle (Luh, 1991). This means there is competition for assimilate supply among grains within a panicle. We focused on this characteristic and tried to clarify the effect of assimilate supply and temperature during ripening period on occurrence of chalky rice grain.

2. Materials and Methods

Plant Materials

Experiments were conducted as pot experiments the research fields on the campus of the College of Agriculture, Ibaraki University Ami-machi, Ibaraki, Japan. Six rice cultivars; Koshihikari, Akitakomachi, Fusaotome, Fusakogane, Haenuki and Sasanishiki were used in this study. These cultivars were used to compare cultivars with different traits for occurrence of chalky rice grain. Ishizaki (2006) previously reported that there were differences among these cultivars in terms of perfect grain rate when the daily temperature was high 20 days after heading.

Rice was cultivated by direct sowing method in 1/5000a Wagner's pots from June to September. Basal fertilizer containing 4 g ammonium sulphate ($(NH_4)_2SO_4$), 4 g superphosphate, and 2 g potassium chloride (KCl) was applied before seeding. The rice seeds were first disinfected by soaking in thiuram benomyl solution for approximately 20 hours, then germination process continued by soaking the seeds in water, and storing at room temperature for 4 days. Seeds were directly sowed as soon as the seeds were germinated (embryo starts to emerge). As top dressing, liquid fertilizer containing 0.95 g ammonium sulphate ($(NH_4)_2SO_4$), 0.25 g superphosphate, and 0.12 g potassium chloride (KCl) per pot diluted with 400 m*l* water was applied two times before heading (50 and 70 days after seeding). The pots were filled with water to approximately 2−3 cm depth 20 days after emergence. All plants were set to be mono stem by removing all emerging tillers during the entire growth period.

Flowering

After we observed heading, flowering was analyzed every 1:00 pm from the next day of heading date. We investigated the order of all grains within a panicle in all cultivars by visual observation. Then the state of the anthers was observed one grain by one grain for all grains within a panicle. Anthers were judged as flowering when an elongate anther was observed. Twenty plants were used as replicates.

Measurements of grain weight and evaluation of grain quality

Plants were harvested at 40 days after heading (DAH), and then the rice samples were dried under room temperature for 2 weeks. Grain weight of all of the brown rice grains within a panicle were measured with an electronic balance. Grain quality was evaluated by eyesight according to the standards of the Ministry of Agriculture, Forestry and Fisheries of Japan. Grain chalkiness was measured by placing the brown rice grain on a white light box to make the grain transparent so the chalky part (white portion inside the grain) could be observed.

Scanning Electron Microscope (SEM) observations

Scanning Electron Microscope type JSM6360A, JEOL, Japan, was used for analyzing the morphological structure of starch accumulation in rice grains. Brown rice grains from each cultivar were used for SEM observations. The rice grains were cut into half with a razor blade, attached to specimen stubs and coated with osmium before observation by SEM.

3. Results and Discussion

As a result, we could observe many chalky rice grains (Fig. 1.) Percentage of imperfect rice grain occurrence was 50.5, 39.5, 32.0, 20.0, 13.0 and 8.5% in Fusaotome, Fusakogane, Akitakomachi, Haenuki, Sasanishiki and Koshihikari, respectively (Fig. 2). Occurrence of white belly rice was high in Fusaotome, Fusakogane and Akitakomachi. Large differences of chalky rice grain occurred in each cultivar, but the factors causing theses differences in the occurrence of chalky rice grain could not be explained. Cultivar showed differences in the number of growing days between heading to sowing, because each average

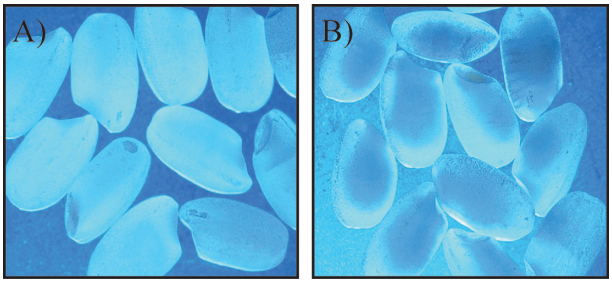

Fig. 1. Appearance of perfect rice grains (A) and chalky rice grains (B). Pictures were taken by a camera with the light source underneath the rice grains.

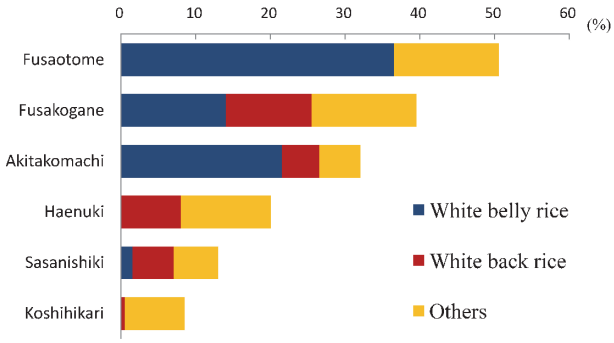

Fig. 2. Percentage of the occurrence of chalky rice grains in six cultivars of Japonica rice.

Table 1. Daily average temparature after heading and brown rice quality

Cultivars	Heading date (Month/days)	Daily Average Temperature (℃)		Perfect graln (%)	Chalky rice grains		
		10 days after heading	20 days after heading		Belly (%)	Back (%)	others (%)
Fusaotome	8/11	29.0	26.9	49.5	36.5	0.0	14.0
Fusakogane	8/12	28.0	26.7	60.5	14.0	11.5	14.0
Akitakomachi	8/14	26.6	26.5	68.0	21.5	5.0	5.5
Haenuki	8/16	26.0	26.3	80.0	0.0	8.0	12.0
Sasanishiki	8/19	24.4	25.6	87.0	1.5	5.5	6.0
Koshihikari	8/21	24.9	25.9	91.5	0.0	0.5	8.0

temperature during initial ripening stage was different depending on cultivars (Table 1). Particularly, the average temperature 10 days after heading was 29.0, 28.0, 26.6, 26.0, 24.4 and 24.9℃ in Fusaotome, Fusakogane, Akitakomachi, Haenuki, Sasanishiki and Koshihikari, respectively. Therefore, cultivar differences could not be clearly defined.

Figure 3 shows the ultrastructure of starch accumulation in the endosperm of rice grains of Fusaotome. In the transparent part, a densely large amyloplast filled with starch granules was observed. On the other hand, in the chalky part, the size of the amyloplast varied from a small size containing only a single starch granule to large sized amyloplasts that contained many starch granules. The shape of the amyloplast also became round and resulted in the creation of numerous empty spaces between amyloplasts. Numerous empty spaces remain among loosely packed starch granules and reflect light, thus appearing chalky. This result agrees with the previous report by (Zakaria *et al.*, 2002).

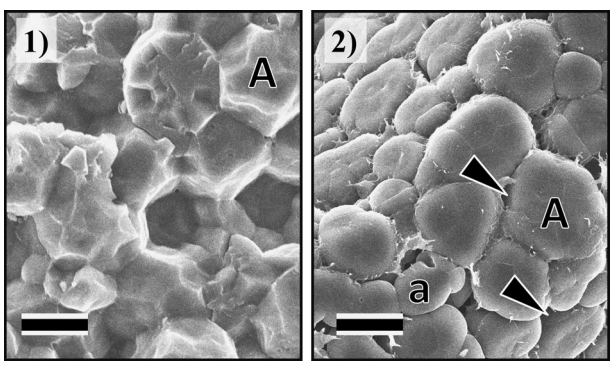

Fig. 3. Ultrastructure of starch accumulation in perfect (1) and chalky (2) rice grains. A: amyloplast, a: small amyloplast, Arrow: empty space, Bar: 10 μm.

There was a significant negative correlation between the days from heading to flowering and rice grain weight in all cultivars, suggesting that rice grain weight was caused by the length of ripening period. There was also a negative correlation between the

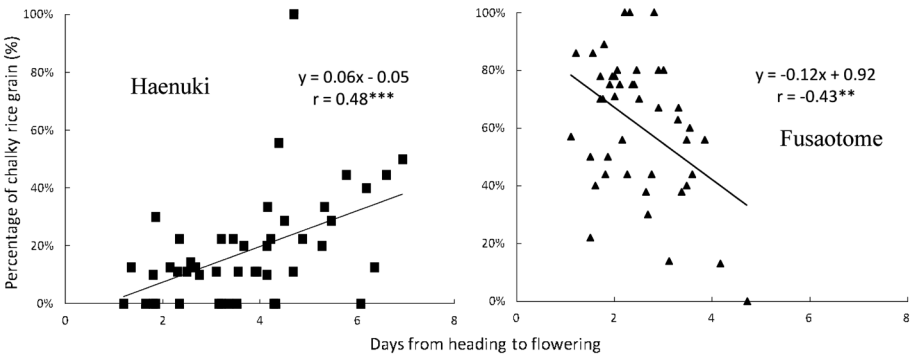

Fig. 4. Relationships between the percentage of chalky rice grains and days from heading to flowering in Haenuki and Fusaotome cultivars.

occurrence of chalky rice grain and days from heading to flowering in Fusaotome, Fusakogane and Akitakomachi. However, a significant positive correlation was found between the occurrence percentage of chalky rice grain and days from heading to flowering in Haenuki and Sasanishiki. Figure 4 shows the relationships between occurrence of chalky rice grain and days from heading to flowering in Fusaotome and Haenuki. For Koshihikari, no significant correlation was found.

As a result of the analysis of all grains within a panicle for each cultivar, it was found that 2 patterns occurred in chalky rice grains. First, the occurrence percentage of chalky rice grain was higher in the early flowering grains. In this case, the occurrence percentage of chalky rice grain was higher in spite of a longer ripening period. Therefore, chalky rice grain did not occur due to a deficit in assimilation products but something else, for example, heat damage. Second, the percentage of chalky rice grains was higher in the later flowering grains and lighter grains. In this case, chalky rice grain likely occurred because of a deficit in assimilation products.

4. Conclusion

The results of this study show that flowering order and daily average temperature at initial ripening stage are related to the occurrence of chalky rice grain. Also, the analyses showed that taking into consideration all of the grains within a panicle is a useful method for determining factors that cause the occurrence of chalky rice grain. However, we could not clearly reveal how the interactions of genetic factors and environmental factors cause the occurrence of chalky rice grain. Therefore, further investigations are necessary to clarify the causes of the occurrence of chalky rice grains.

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