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REGULAR ARTICLE

Occurrence of perfect and imperfect grains of six japonica rice cultivars as affected by nitrogen fertilization

Jiangfang Qiao · Zhenghui Liu · Shanyu Deng · Huifeng Ning · Xiaoyu Yang · Zhaomiao Lin · Ganghua Li · Qiangsheng Wang · Shaohua Wang · Yanfeng Ding

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Abstract This study aims to quantify nitrogen (N) effect on occurrence of perfect rice kernel (PRK) and imperfect grains which includes white-belly rice kernel (WBRK), white-core rice kernel (WCRK), green rice kernel (GRK), opaque rice kernel (ORK), and other imperfect grains (OTHERS). Two-year field experiments involving six japonica rice cultivars and seven N treatments were performed. The structural differences between white-belly and white-core tissues were compared using scanning electron microscope. Averaged over cultivars, grain yield increased progressively with N rate. PRK increased with N rate in 2008, but decreased with increased N rate in 2009. WBRK and WCRK decreased as N rate increased for both years. High N input resulted in higher occurrence of GRK and OTHERS for both years. Most starch granules in white-belly tissues are intact and surrounded by globular protein bodies, with many air spaces between them; while in white-core tissues, starch granules are easily broken into many single granules and no protein bodies are visible. Our results suggest that N has suppressing influence on chalky grains but favorable effect on other imperfect grains, and indicate different mechanism between WBRK and WCRK.

Keywords Appearance quality · White-belly rice kernel · White-core rice kernel · Nitrogen · Japonica rice

Introduction

Quality of grain is an important component in the marketability of rice products and hence affects the profitability of rice growers. A continuous improvement in rice quality is necessary to meet the growing global demand for high quality (Fitzgerald et al. 2009). Rice, unlike most other cereals, is consumed as a whole grain. Therefore, general appearance including grain size and shape, percentage of chalkiness rice kernel, and ratio of perfect to imperfect grain is of great importance in rice production and consumption.

According to the grading standard in Japan, brown rice is classified into perfect and imperfect kernels (Hoshikawa 1993). The perfect rice kernel (PRK) is the one that normally and perfectly ripens and has the shape of brown rice (grain shape) characterizing the cultivar. By contrast, brown rice with some abnormality or defect in grain shape, size, color, and luster, is collectively called imperfect rice kernel. Imperfect rice has a variety of types: chalky rice, cracked rice,

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J. Qiao · Z. Liu · S. Deng · H. Ning · X. Yang · Z. Lin · G. Li · Q. Wang · S. Wang · Y. Ding (✉)
College of Agronomy, Nanjing Agricultural University,
Nanjing 210095, China
e-mail: dingyf@njau.edu.cn

notched-belly rice, opaque rice, green rice, rusty rice, and so forth. Depending on the position of opaque part in grains, chalky rice is divided into different types including white-belly rice, white-core rice, milky-white rice, white-base rice, and white-back rice. White-belly and white-core rice, which have opaque area on the ventral side and in the center part of grains, respectively, are the major two types of chalky rice (Hoshikawa 1993). Although chalk is an undesirable trait because of its detrimental effect on head rice yield, white belly rice and white core rice can still be treated as almost the same as perfect rice in quality (Hoshikawa 1993). By contrast, other types of imperfect rice including opaque rice, green rice, and rusty rice are improper for food and will be discarded during rice milling.

Rice plays a critical role in China's food security. A continuous improvement in rice production is a challenging task to keep abreast of the population growth. China's rice agriculture is now characterized by high input of fertilizer, water, and pesticide and high output of grains. In Jiangsu province, for example, the averaged grain yield is about 8.0 t/ha and the N input is as high as about 330 kg/ha provincially (Xue and Yang 2008). Overuse of N fertilizer has caused substantial N loss (especially nitrate leaching and ammonia volatilization) and soil acidification (Guo et al. 2010). Thus, many studies have been conducted to develop optimized N fertilization methods aimed at both high yield and high N use efficiency (NUE) in China. Among them, a method called high fertilization at panicle initiation (PI) stage, i.e. reducing the N rate as basal before transplanting whereas increasing the topdressing rate at panicle initiation stage, has been recommended recently (Ling et al. 2005). This fertilization mode demonstrated higher effective tiller number, larger panicle with more grains, and consequently higher grain yield and high NUE, in comparison with the conventional method where almost all the fertilizer is applied as basal dressing. However, little is known concerning the effect of this N management on the occurrence of different types of grains.

During the investigation of N influence on grain biochemical composition in 2007 (Ning et al. 2009), we found that samples from high N rate treatments contained higher ratio of imperfect grains than those of low N treatments. This finding indicates that farmers could not benefit from the elevated grain yield by high N rate, since the imperfect grains will be removed or screened during milling. Thus we conducted 2-year field experiments in 2008 and 2009 to quantify the N effect on the occurrence of different types of grains, with emphasis on white-belly and white-core grains. In addition, we also investigated the structure of chalky tissues by scanning electron microscope (SEM) to determine the anatomical differences between white-belly and white-core tissues.

Materials and methods

The filed experiment was performed at Jiangning Experimental Station of Nanjing Agricultural University (31°56′39″N, 118°59′13″E) in 2008 and at Danyang Experimental Station (31°54′31″N, 119°28′21″E) in 2009. The design was a randomized split-plot design with seven N treatments split for six genotypes. There were four and three replications in 2008 and 2009, respectively. The size of main plot was 5.0 m×4.0 m in 2008 and 2009, and a ridge covered plastic film was constructed between plots to avoid seepage. Six japonica rice cultivars differing in panicle size, heading date, and cooking quality were used, as reported by Ning et al. (2010). In 2008, average heading dates of the seven treatments were as follows: Zaofeng9 and Xudao4, August 20; Wuyujing3 and Ningjing2, August 28; Ningjing1 and 9522, September 4. In 2009, Zaofeng9 and Xudao4 headed on August 24, and the other four cultivars headed nearly on the same day as that in 2008.

Seven N treatments were conducted: (1) CK, no N fertilizer was applied during the whole growth stage, except that P and K fertilizer was applied before transplanting; (2) LN82 and LN55, low N rate (90 kg/ha); (3) MN82 and MN55, moderate N rate (180 kg/ha); (4) HN82 and HN55, high N rate (270 kg/ha). Note that fertilization modes of 55 and 82 mean the ratio of basal/topdressing fertilizer is 5:5 and 8:2, respectively.

The soil fertility and agronomic practices at Jiangning in 2008 were described by Ning et al. (2010). The soil type at Danyang is clay soil, containing 1.10 g/kg total N, 12.23 mg/kg available P, and 119.41 mg/kg exchangeable K. In 2008, rice was sown in seedbeds on May 25, and transplanted on June 28. In 2009, rice was sown in seedbeds on May 22, and transplanted on June 29. At maturity, about 100 panicles with similar maturity were harvested in each replication. The samples were naturally dried and dehulled.

As shown in Fig. 1, brown rice was categorized into six groups according to Hoshikawa (1993): perfect rice kernel (PRK), white-belly rice kernel (WBRK), white-core rice kernel (WCRK), green rice kernel (GRK), opaque rice kernel (ORK), and other imperfect grains such as misshapen grains (OTHERS). For observation of endosperm cross-section, rice grains were dried completely under low pressure and cut across the short axis with razor blade. The surface was sputter-coated with gold in vacuum and observed by SEM (Hitachi S-3000 N) at an accelerating voltage of 15 Kv. Representative grains of PRK, WBRK, and WCRK of the six cultivars under CK treatment were examined and photographed.

Samples were analyzed in triplicate and mean values were used for comparisons. Variance analysis was performed using the Data Processing System (DPS, Institution of Agricultural Entomology, Zhejiang University). Means were compared by the least significant difference (LSD) test ($P \leq 0.05$).

Results

Yield performance

As shown in Table 1, effect of N fertilizer and cultivar are both significant for grain yield. Averaged over cultivars, grain yield increased progressively with N rate up to 9002.3 kg/ha at N rate of 270 kg/ha (Table 2). Increasing the ratio of top-dressing to basal fertilizer didn't obtain significant higher grain yield for low and medium N rate for both years, but resulted in higher grain yield for high N rate in 2009. This result is in disagreement with our previous finding that higher ratio of

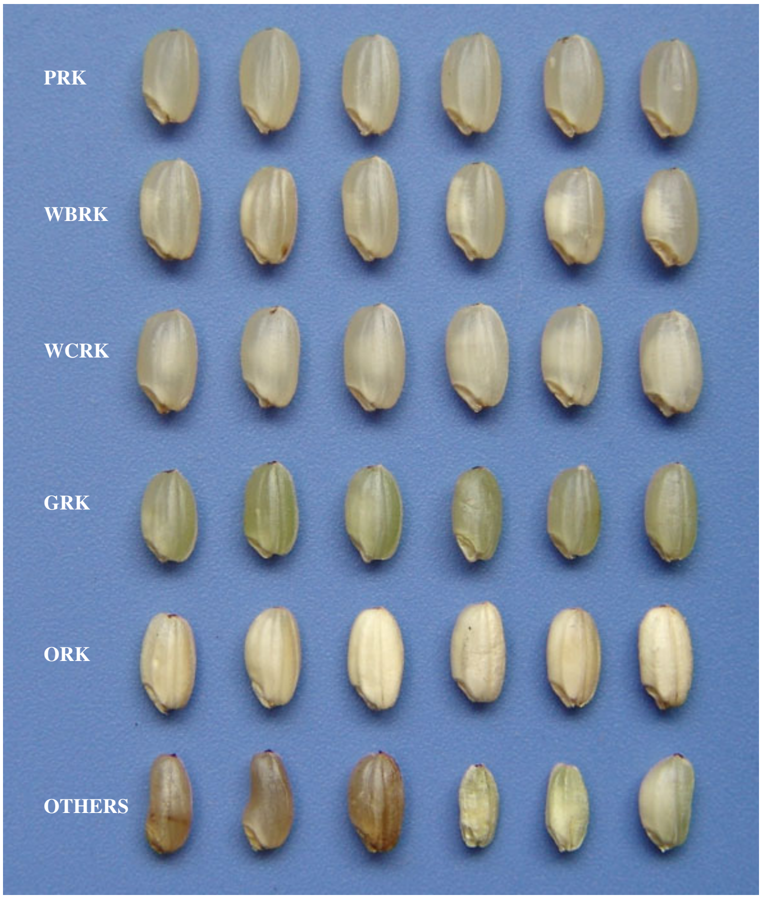

Fig. 1 Category of perfect and imperfect rice grains. Brown rice was divided into six groups: *PRK* perfect rice kernel; *WBRK* white-belly rice kernel; *WCRK* white-core rice kernel; *GRK* green rice kernel; *ORK* opaque rice kernel; and other imperfect grains such as misshapen grains (OTHERS). The cultivar used for presentation is 9522

Table 1 Variance analysis for the N and genotype effect on grain yield, occurrence of perfect and imperfect grains

Year	Source of variation	df	Grain yield	PRK[a]	WBRK	WCRK	GRK	ORK	OTHERS
2008	G	5	37306.65**	261.42**	231.65**	196.3**	94.71**	42.66**	8.53**
	N	6	179225.78**	5.01**	2.84*	3.87**	3.89**	5.72**	8.35**
	G×N	30	9588.72**	3.46**	3.99**	2.47**	1.52	2.18**	1.80*
2009	G	5	61160.55**	83.23**	48.17**	20.36**	64.6**	149.92**	11.04**
	N	6	54403.70**	3.84**	7.21**	6.33**	33.68**	0.47	8.29**
	G×N	30	7888	1.51	2.58**	1.66*	1.31	1.37	1.57

Data presented are mean squares.

[a] *PRK* perfect rice kernel; *WBRK* white-belly rice kernel; *WCRK* white-core rice kernel; *GRK* green rice kernel; *ORK* opaque rice kernel; *OTHERS* the remaining grains.

*, ** significant at 0.05 and 0.01 probability level, respectively.

topdressing to basal increases grain yield for moderate N rate but not for high N rate (Ning et al. 2009). This inconsistency might be attributed to differences between genotypes and N treatments in the two studies.

Significant genotypic variations in grain yield were detected among the cultivars examined. Pooled across N treatments, grain yield was highest for 9522 and lowest for Xudao4 and Wuyujing3 in 2008 and 2009, respectively (Table 2). In addition, genotypic differences in yield performance response to N treatments existed. For example, Xudao4, the well-known super-rice cultivar for its high yield potential, was less sensitive to N treatments, with a coefficient of variation (CV) being the lowest among the six cultivars for both years. By contrast, grain yield of another Chinese super-rice cultivar, Ningjing1, was more affected by N fertilizer, showing the higher CV among the six cultivars for both years.

Table 2 Grain yield of the six cultivars for the seven N treatments in 2008 and 2009

Year	Cultivar	CK[a]	LN82	LN55	MN82	MN55	HN82	HN55	Mean	CV
2008	9522	5426.3c	7860.0b	7936.4b	10360.5a	9120.0ab	9342.2a	9545.3a	8513.0a	19.08
	Ningjing1	4759.4d	6529.1c	7097.1bc	7951.2b	7319.4bc	9621.9a	10185.6a	7637.7cd	24.16
	Ningjing2	5624.0d	7471.4bc	7247.0c	8677.2ab	7782.5bc	9801.8a	8751.9ab	7908.0bc	16.93
	Wuyunjing3	5430.5d	8098.5ab	6863.4bc	6353.6cd	8186.3ab	8463.9a	8079.3ab	7353.6de	15.65
	Xudao4	5031.2b	6960.6a	7009.4a	7250.3a	7570.4a	7979.6a	7220.0a	7003.1e	13.39
	Zaofeng9	5241.6e	7875.3cd	7046.3d	8716.1bc	9548.0ab	8434.8bc	10232.0a	8156.3ab	20.30
	Mean	5252.1d	7465.8c	7199.9c	8218.2b	8254.4b	8940.8a	9002.3a	7761.9	
2009	9522	8438.0bc	8497.5bc	8804.3bc	9944.3ab	7672.1c	8934.5bc	11079.9a	9052.9a	12.41
	Ningjing1	6489.3c	6564.6c	8279.1abc	7740.2bc	7075.2bc	8831.4ab	10059.3a	7862.7bc	16.54
	Ningjing2	6380.7b	8307.6ab	7330.2ab	8266.1ab	9167.6a	9054.8a	9432.2a	8277.0b	13.26
	Wuyunjing3	6216.0b	5971.8b	6867.9b	6122.1b	7452.2ab	6874.1b	9076.8a	6940.1d	15.53
	Xudao4	8359.4a	8260.2a	9111.0a	7788.9a	9173.1a	8456.6a	9009.5a	8594.1a	6.03
	Zaofeng9	6091.4c	6016.5c	6782.3bc	8938.4a	6392.4c	7806.0abc	8567.9ab	7227.8cd	16.67
	Mean	6995.8d	7269.7cd	7862.5bc	8133.3b	7822.1bc	8326.2b	9537.6a	7992.4	

Data for the seven N treatments within a line followed by a different letter are significantly different ($P<0.05$). However, data within column of "Means" followed by a different letter are significantly different ($P<0.05$).

[a] *CK* control of fertilizer treatments; *CV* coefficient of variation; *HN* high nitrogen rate of 270 kg/ha; *LN* low nitrogen rate of 90 kg/ha; *MN* moderate nitrogen rate of 180 kg/ha; 82, 80% nitrogen fertilizer applied as basal while 20% as topdressing; 55, nitrogen fertilizer applied equally between basal and topdressing.

N and genotype effect on the occurrence of perfect and imperfect grains

Variance analysis showed significant effect of N and genotype on the occurrence of perfect and imperfect grains both in 2008 and 2009, except for the effect of N on ORK in 2009 (Table 1).

Genotypic differences in the percentages of perfect and imperfect grains were found among the cultivars examined. For example, Ningjing2 contained higher percentage of PRK, whereas Wuyujing3 had lower percentage. And percentages of WBRK in 9522, Wuyujing3, and Zaofeng9 were consistently higher than those of Ningjing2 and Xudao4 for both years.

Figure 2 shows clearly that high N input resulted in higher occurrence of imperfect grains, especially GRK and OTHERS. PRK is the largest group, accounting for 46.92% and 61.40% of the total rice grains in 2008 and 2009, respectively. As presented in Tables 3 and 4, pooled across cultivars, PRK increased with N rate in 2008, but decreased with N rate in 2009. However, one of the six cultivars, Zaofeng9, showed opposite trend in 2008, with its PRK decreasing with N rate. For the three N rates, high ratio of topdressing to basal fertilizer showed no significant effect on PRK occurrence in 2008 and 2009, except for the low and high N rate in 2008.

Percentages of the two types of chalky grains, WBRK and WCRK, varied with growth years, with the year of 2008 having larger values than that of 2009 (Tables 3 and 4). When averaged across cultivars, percentages of WBRK and WCRK decreased as N rate increased for both years, indicating that N has a suppressing influence on chalkiness formation. For the three N rates, high ratio of topdressing to basal fertilizer showed no significant effect on WBRK and WCRK occurrence in 2008 and 2009, except for the effect of high N rate on WBRK in 2008. Further, significant genotypic variations in response of WBRK to N fertilization were detected. N showed a suppressing effect on occurrence of WBRK for 9522, Wuyujing3, and Zaofeng9, whereas exhibited a promoting effect on that for Ningjing1 in 2008.

GRK and OTHERS are the majority of the imperfect grains, being about one-fourth of the total

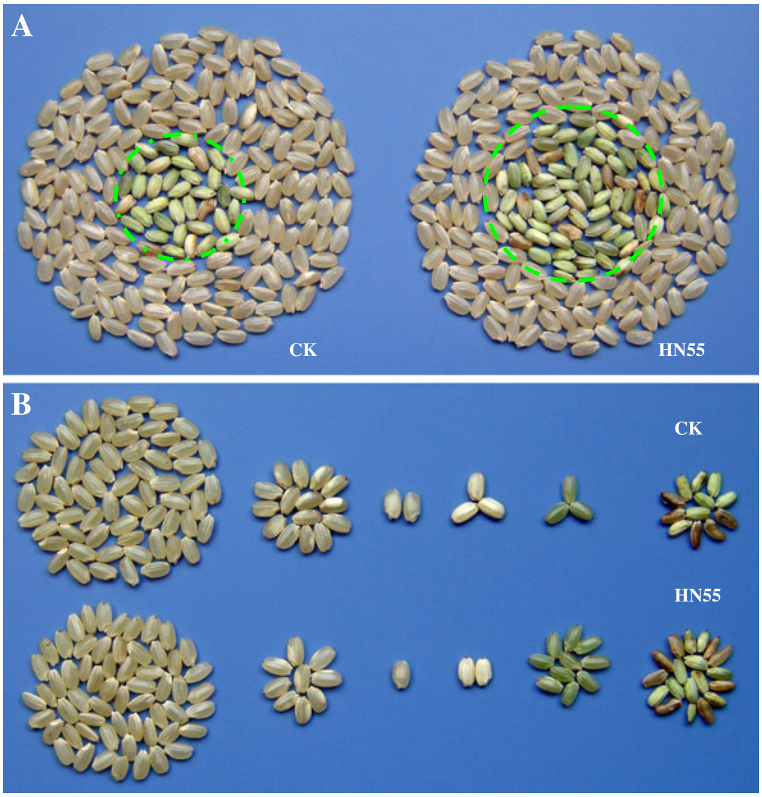

Fig. 2 Comparison of percentage of perfect and imperfect grains between CK and HN treatments. **a** Grains within the green circle with broken line are GRK, ORK, and OTHERS. **b** The number of each type grain is average across the six cultivars grown in 2009. The cultivar used for presentation is 9522. CK, no N fertilizer was applied; HN55, high N rate (270 kg/ha), with the ratio of basal/topdressing fertilizer being 5:5

Table 3 Occurrence of perfect and imperfect grains under seven N treatments in 2008

Grain types	Cultivar	CK	L82	L55	M82	M55	H82	H55	Mean	CV
PRK	9522	51.92a	46.25a	48.67a	47.00a	46.96a	47.17a	54.13a	48.87b	6.13
	Ningjing1[a]	17.13d	30.38c	41.13ab	42.42ab	44.58ab	37.71b	45.88a	37.03c	27.49
	Ningjing2	66.67a	69.29a	68.46a	67.46a	68.96a	58.54b	67.25a	66.66a	5.56
	Wuyunjing3	30.96a	29.33a	30.88a	27.92a	34.92a	28.71a	32.54a	30.75e	7.84
	Xudao4	62.08a	60.71a	65.92a	63.46a	65.67a	65.33a	65.96a	64.16a	3.29
	Zaofeng9	39.29a	29.50b	34.33ab	34.00ab	34.33ab	32.61ab	34.38ab	34.06d	8.52
	Mean	44.68b	44.24b	48.23a	47.04ab	49.24a	45.01b	50.02a	46.92	
WBRK	9522	26.75a	26.38a	23.58a	23.04a	19.71ab	15.21bc	11.71c	20.91c	27.21
	Ningjing1	3.25e	8.50de	11.50cd	16.08bc	18.88ab	24.21a	18.88ab	14.47d	49.41
	Ningjing2	8.88a	9.21a	8.63a	7.21a	8.96a	13.42a	8.17a	9.21e	21.40
	Wuyunjing3	46.46ab	48.88a	45.71ab	47.92ab	38.96c	41.67bc	35.67c	43.61a	11.36
	Xudao4	10.88a	10.38a	10.46a	9.96a	5.83a	8.54a	8.50a	9.22e	19.12
	Zaofeng9	30.50a	29.33ab	27.75ab	29.42ab	28.71ab	26.89ab	23.00b	27.94b	8.86
	Mean	21.12a	22.11a	21.27a	22.27a	20.18ab	21.66a	17.66b	20.89	
WCRK	9522	1.50b	3.79ab	3.96ab	2.96ab	4.67a	3.25ab	2.75ab	3.27b	31.12
	Ningjing1	17.21a	15.25ab	16.54a	11.17cd	12.88bc	9.33d	10.58cd	13.28a	23.30
	Ningjing2	3.08ab	1.42b	3.04ab	1.13b	0.88b	4.29a	1.54b	2.20c	58.11
	Wuyunjing3	2.13a	2.75a	1.92a	2.17a	1.33a	0.54a	0.58a	1.63c	51.65
	Xudao4	0.25a	0.29a	0.25a	0.04a	0.04a	0a	0.17a	0.15d	80.90
	Zaofeng9	2.50a	2.44a	1.67a	1.63a	0.92a	1.39a	1.04a	1.66c	37.55
	Mean	4.45a	4.32a	4.56a	3.18b	3.45ab	3.13b	2.78b	3.70	
GRK	9522	6.04b	8.96ab	8.04ab	6.71ab	7.88ab	10.67a	9.50ab	8.26c	19.38
	Ningjing1	0.88c	1.92bc	2.58bc	5.13ab	4.92abc	7.88a	5.83ab	4.16 d	59.18
	Ningjing2	7.29a	7.13a	5.75a	8.54a	6.58a	7.17a	5.71a	6.88c	14.3
	Wuyunjing3	2.63b	4.29ab	5.04ab	4.96ab	6.08ab	6.71ab	8.04a	5.39 d	32.45
	Xudao4	11.92ab	13.25ab	11.21b	12.50ab	15.75a	13.50ab	11.88ab	12.86b	11.71
	Zaofeng9	13.96c	21.17a	17.33abc	16.67bc	19.54ab	15.00c	17.33abc	17.29a	14.33
	Mean	7.12c	9.45ab	8.33bc	9.09ab	10.13a	10.16a	9.72ab	9.14	
ORK	9522	2.25ab	2.38a	2.13ab	0.92ab	0.50b	0.83ab	0.58ab	1.37b	61.41
	Ningjing1	4.25b	6.25a	4.08b	4.79ab	1.92c	3.79b	1.92c	3.86a	40.04
	Ningjing2	0.29a	0.50a	0.25a	0.29a	0.04a	0.67a	0.17a	0.32c	66.24
	Wuyunjing3	4.75a	1.50b	1.21b	0.67b	0.38b	0.96b	0.17b	1.38b	113.06
	Xudao4	0a	0a	0a	0a	0a	0.04a	0a	0.02c	183.59
	Zaofeng9	0.08a	0.11a	0.58a	0.08a	0.08a	0a	0.38a	0.19c	112.71
	Mean	1.94a	1.80ab	1.38abc	1.13bcd	0.49d	1.05cd	0.54d	1.19	
OTHERS	9522	11.54b	12.25b	13.63b	19.38a	20.29a	22.88a	21.33a	17.33a	27.15
	Ningjing1	17.63a	13.00ab	11.42b	13.92ab	14.33ab	15.33ab	16.33ab	14.57b	14.27
	Ningjing2	13.79a	12.46a	13.88a	15.38a	14.58a	15.92a	17.17a	14.74b	10.57
	Wuyunjing3	13.08c	13.25c	15.25c	16.38bc	18.33abc	21.42ab	23.00a	17.24a	22.43
	Xudao4	14.88a	15.29a	12.17a	14.04a	12.33a	12.58a	13.5a	13.54b	9.23
	Zaofeng9	13.67b	17.44b	18.33b	18.21b	16.42b	24.11a	23.88a	18.87a	20.34
	Mean	14.10b	13.95b	14.11b	16.22b	16.05b	18.71a	19.20a	16.05	

[a] In 2008, high occurrence of white-back and combined chalkiness was observed for Ningjing1, and the data are not shown in this table.

Table 4 Occurrence of perfect and imperfect grains under seven N treatments in 2009

Grain types	Cultivar	CK	L82	L55	M82	M55	H82	H55	Mean	CV
PRK	9522	56.17ab	57.75ab	65.75a	59.33ab	56.50ab	56.42ab	54.00b	57.99d	6.53
	Ningjing1	69.50a	66.50ab	68.17ab	62.92ab	64.58ab	62.58ab	58.92b	64.74c	5.62
	Ningjing2	87.67a	79.83ab	75.75bc	72.17bc	68.83c	68.50c	68.50c	74.46a	9.70
	Wuyunjing3	43.42ab	37.08b	45.83ab	40.83ab	39.50ab	43.42ab	48.17a	42.61e	8.89
	Xudao4	77.00a	73.92ab	71.00abc	67.92abc	67.33abc	66.08bc	63.42c	69.52b	6.79
	Zaofeng9	59.00a	60.25a	57.00a	58.33a	59.33a	58.67a	61.08a	59.09d	2.24
	Mean	65.46a	62.56abc	63.92ab	60.25bc	59.35c	59.28c	59.02c	61.40	
WBRK	9522	19.08a	10.08b	6.17b	7.92b	8.25b	8.83b	6.83b	9.59c	45.59
	Ningjing1	8.25a	9.50a	9.83a	11.58a	10.50a	10.42a	10.08a	10.02c	10.20
	Ningjing2	4.08a	4.08a	4.08a	5.33a	5.00a	6.83a	4.17a	4.80e	21.53
	Wuyunjing3	28.50a	19.67b	15.00bc	16.33bc	14.50bc	18.17bc	13.33c	17.93a	28.70
	Xudao4	6.75a	7.67a	7.83a	8.83a	7.83a	6.50a	6.08a	7.36d	12.96
	Zaofeng9	20.58a	13.00b	14.25b	12.25b	10.75b	9.83b	11.00b	13.09b	27.65
	Mean	14.54a	10.67b	9.53b	10.37b	9.47b	10.10b	8.58b	10.47	
WCRK	9522	0.42a	0a	0a	0.08a	0.08a	0.17a	0.08a	0.12c	122.26
	Ningjing1	5.75a	2.92b	2.08b	1.58b	1.25b	2.42b	1.17b	2.45a	64.64
	Ningjing2	0.42a	0.83a	0.42a	0.50a	0.58a	0.92a	0.67a	0.62bc	31.76
	Wuyunjing3	0.92a	1.33a	0.58a	0.58a	0.42a	0.17a	0.33a	0.62bc	63.44
	Xudao4	2.83a	0.50b	1.00b	0.75b	0.75b	0.58b	0.67b	1.01b	80.81
	Zaofeng9	4.08a	1.08b	1.75b	1.58b	1.58b	2.42b	2.58ab	2.15a	46.21
	Mean	2.40a	1.11b	0.97b	0.85b	0.78b	1.11b	0.92b	1.16	
GRK	9522	8.33a	14.33a	13.83a	15.00a	16.25a	14.67a	15.25a	13.95a	18.59
	Ningjing1	1.58d	6.75bc	5.83c	9.67ab	7.58abc	10.25a	10.75a	7.49c	42.67
	Ningjing2	1.17c	5.58b	5.58b	7.08ab	9.75a	9.17a	8.00ab	6.62c	43.74
	Wuyunjing3	2.92b	9.17a	10.33a	11.17a	8.92a	9.25a	8.83a	8.66b	30.84
	Xudao4	1.67b	4.67ab	4.08ab	4.42ab	5.00a	5.25a	5.83a	4.42d	30.31
	Zaofeng9	0.75c	5.67b	8.08ab	9.33a	9.92a	9.00a	8.92a	7.38c	43.77
	Mean	2.74c	7.70b	7.96b	9.45a	9.57a	9.60a	9.60a	8.09	
ORK	9522	0.42a	0.08a	0a	0.17a	0.25a	0.17a	0.08a	0.17d	82.45
	Ningjing1	0.08a	0.33a	0.17a	0.33a	0.08a	0.25a	0.50a	0.25d	61.34
	Ningjing2	0.17a	0.17a	0.25a	0.17a	0.08a	0a	0.33a	0.17d	64.13
	Wuyunjing3	12.75a	10.58ab	10.00bc	7.67c	10.25b	9.33bc	8.08bc	9.81a	17.30
	Xudao4	3.50a	1.67a	2.92a	3.08a	3.00a	2.67a	2.33a	2.74c	21.66
	Zaofeng9	2.33b	3.67b	4.08b	6.50a	3.67b	3.92b	3.58b	3.96b	31.64
	Mean	3.21a	2.75a	2.90a	2.99a	2.89a	2.72a	2.48a	2.85	
OTHERS	9522	15.58b	17.75ab	14.25b	17.50ab	18.67ab	19.75ab	23.75a	18.18a	16.89
	Ningjing1	14.83a	14.00a	13.92a	13.92a	16.00a	14.08a	18.58a	15.05b	11.51
	Ningjing2	6.50c	9.50bc	13.92ab	14.75ab	15.75ab	14.58ab	18.33a	13.33b	30.02
	Wuyunjing3	11.50c	22.17ab	18.25b	23.42ab	26.42a	19.67b	21.25ab	20.38a	23.15
	Xudao4	8.25d	11.58cd	13.17bcd	15.00bc	16.08abc	18.92ab	21.67a	14.95b	30.11
	Zaofeng9	13.25a	16.33a	14.83a	12.00a	14.75a	16.17a	12.83a	14.31b	11.64
	Mean	11.65d	15.22c	14.72c	16.10bc	17.95ab	17.20abc	19.40a	16.03	

grains (Tables 3 and 4). Increasing N exhibited promoting effect on the occurrence of GRK and OTHERS, and high ratio of topdressing to basal fertilizer showed no significant effect for both years.

ORK was the smallest group of rice grains, being only about 2% of the total grains. In 2008, ORK varied significantly based on N treatments, with high N rate producing lower percentage of ORK. In 2009, no response to N treatment was detected for ORK (Tables 3 and 4).

SEM of white-belly and white-core grains

As shown in Fig. 3, starch granules in the dorsal, ventral, and central parts of perfect grain are tightly packed with no air space among them, whereas those of the white-belly and white-core tissues are round in shape and loosely packed with many intergranular air spaces. We further compared the structural differences between white-belly and white-core tissues. In white-belly tissues, most of starch granules are intact and surrounded by globular protein bodies, with many air spaces between them (Fig. 4). This result is in agreement with that reported by Tashiro and Ebata (1979). However, the structure of white-core tissues differs with that of white-belly, with starch granules being easily to be broken into many single granules and no protein bodies being observed (Fig. 5). Lisle et al. (2000) also observed the similar structure of chalky tissues by SEM, but the authors didn't present more information on the type of chalkiness.

Discussion

N effect on grain yield

Nitrogen is the most yield-limiting nutrient in rice production, and proper N management is essential for optimizing rice grain yields (Bond et al. 2008). For a given N rate, the recommended new N fertilization that reduces basal application but increases topdressing application had advantages of more panicles and more grains per panicle over the conventional N application pattern that applies almost all the fertilizer as basal (Ling et al. 2005; Xue and Yang 2008). However, in the present study, this method of high fertilization at PI stage didn't show favorable effect on grain yield except for high N rate in 2009, partially due to its insignificant effect on panicle numbers per area and grain number per panicle, as shown in Table S1 and S2 (Online Resource 1). Therefore, the application of this new method to rice production needs to be reconsidered.

Generally, grain yield is calculated by multiplying panicle number per area, filled grain number per panicle, and grain weight. N application can increase both panicle number per area and filled grain number per panicle (Bond et al. 2008; Ghobrial 1980; Koutroubasa and Ntanos 2003). But a considerable part of grains produced under high N rate, being about 25%, are imperfect grains like GRK and OTHERS, which will be discarded during milling and can not be used for human consumption (Fig. 2). Similarly, Sasahara and Itoh (1989) reported that the increase of N level increased number of spikelets per panicle but reduced ripening percentage in all cultivars used. And Bond et al. (2008) found that rough grain yield increased with N rate, but the head rice yield didn't response to it. However, Bond et al. (2008) didn't present an explanation of their result. We speculated that it could be associated with the high ratio of imperfect grains that produced by high N treatments. Given that most of rice is consumed as whole grain (milled rice), it is the milled grain yield or head rice yield not the rough grain yield that is of economic significance for rice producers. Our results suggest that future N strategies should focus on increasing head rice yield as well as grain yield. On the other hand, much attention needs to be paid on the large numbers of imperfect grains, especially measuring their chemical compositions to evaluate their nutritional properties and potential as animal feeds.

N effect on imperfect rice kernel

Imperfections in rice grain have complex physiological foundation (Taira 1995). GRK occurs when rice is harvested and dried while chloroplasts still remain in the pericarp. Most ORKs are caused by a temporary retardation in the grain growth under unfavorable ripening conditions immediately after the flowering. For the six cultivars used in this study, tapered rice kernel and other misshapen rice kernel are the majority of the type of OTHERS. However, little attention has been paid on the physiological foundation of these imperfect grains.

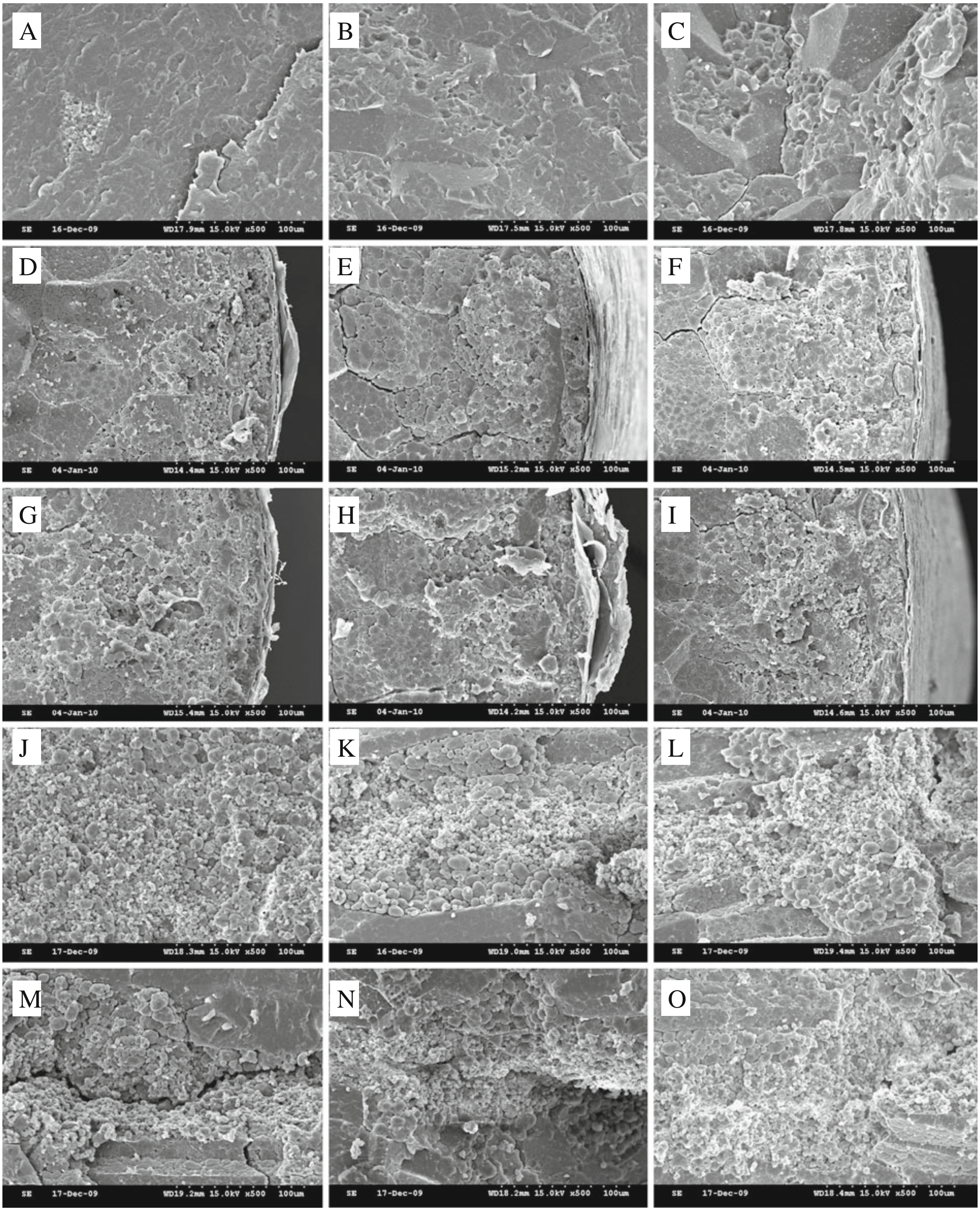

Fig. 3 Scanning electron microscopy (SEM) image of perfect, white-belly, and white-core rice kernels. **a**, **b**, and **c**, dorsal, ventral, and central part of perfect rice grains of Ningjing1; **d**, **e**, **f**, **g**, **h**, and **i**, white-belly of 9522, Ningjing1, Ningjing2, Wuyujing3, Xudao4, and Zaofeng9, respectively; **j**, **k**, **l**, **m**, **n**, and **o**, white-core of 9522, Ningjing1, Ningjing2, Wuyujing3, Xudao4, and Zaofeng9, respectively. Samples from field experiments at Danyang in 2009 were used

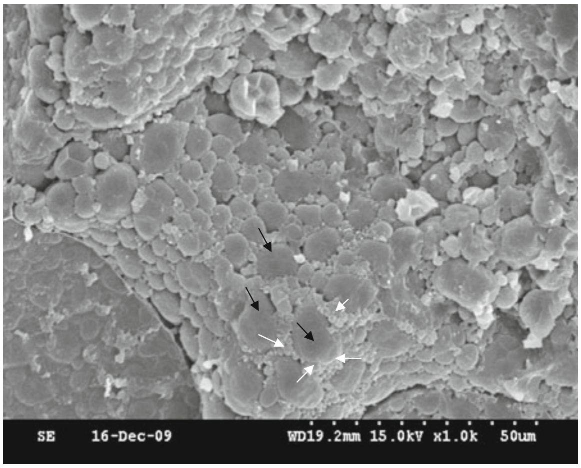

Fig. 4 SEM image of white-belly rice kernels. Black arrows indicate starch granules, and white arrows indicate protein bodies accumulated around starch granules. Note that there are air spaces between starch granules and protein bodies. Grains used are from CK treatment of Wuyujing3 at Danyang in 2009

By contrast, the underlying mechanism of chalky grains has been studied extensively. Starch granules in translucent areas of grains are bigger and more tightly packed than the small loosely packed granules in chalky areas of the grain. Because of this, many studies approach chalk by focusing on processes of starch accumulation (Fitzgerald et al. 2009). It was found to be associated with the insufficient supply of nutrients to the developing endosperm from source organs, the reduced ability to synthesize starch in the endosperm, or the degradation of starch during ripening by α-amylase (Ishimaru et al. 2009; Yamakawa et al. 2007). However, none provide clear guidance towards genetic or biochemical processes underlying chalkiness (Fitzgerald et al. 2009).

Protein bodies account for about 8% of the endosperm's weight, and accumulate between starch granules. However, the role of proteins in the formation of chalky grains is not yet very well known. The SEM images clearly showed the incomplete accumulation of protein body in white-belly tissues, with air spaces between starch granules (Fig. 4). This result indicated that the incomplete accumulation of protein body is another explanation of WBRK formation.

Previous and our result showed that percentage of WBRK decreased with the increase of N rate (Tashiro and Ebata 1979; McClung 2004). It was found that N application can enhance the accumulation of protein bodies in the lateral part and central part of grains, thus occupying the space between unpacked starch granules (Leesawatwong et al. 2005; Zakaria et al. 2000). These findings in combination with our SEM images indicate that the suppressing effect of N on WBRK could be partially attributed to the complete accumulation of protein bodies that can fill the air spaces between starch granules. However, few studies have been conducted to validate this hypothesis so far.

Growing environment had substantial influence on the chalkiness occurrence, with high temperatures during ripening stage showing marked effect on the occurrence of chalky grains (Lisle et al. 2000). Current climate models predict that mean global temperature will continue to increase (Fitzgerald et al. 2009), and high summer temperature has exacerbated the problem of grain injury in Japan in recent years, resulting in serious deterioration of the appearance quality of brown rice (Kobayashi et al. 2007; Tabata et al. 2007). However, N fertilization, a major agronomical practice in rice production, showed a favorable effect of decreasing the ratio of chalky grains. Our results suggest the potential of N application for coping with the adverse effect of the forecasted global warming on rice agriculture.

Note that WBRK of one cultivar, Ningjing1, response differently to N rate compared with that of the other five cultivars, with N application increasing

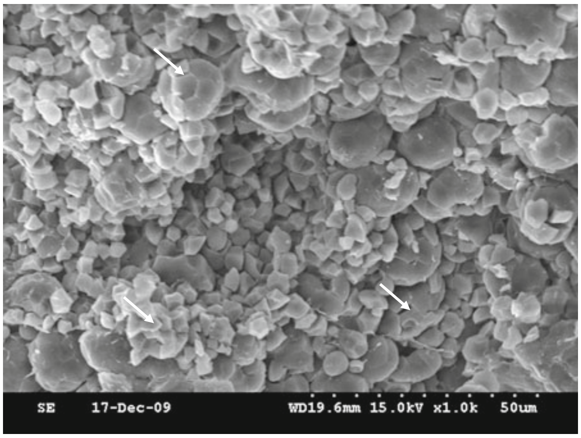

Fig. 5 SEM image of white-core rice kernels. Compound starch granules (white arrows) are easily to be broken into many single granules by the mechanical stress, resulting in a less ordered and loosely packed structure of the chalky part. Further, no protein bodies were visible in white-core tissues. Grains used are from CK treatment of Wuyujing3 at Danyang in 2009

the occurrence of WBRK. This result suggests that N strategy aimed at lower rate of WBRK should vary with genotypes. However, the underlying mechanism of this genotypic difference in response of WBRK to N is still unknown.

Comparison of WBRK and WCRK

WBRK and WCRK are the major two forms of chalky grains for China's japonica rice. It was presumed that the mechanism of WBRK occurrence was different from that of WCRK occurrence. White-core grains have chalkiness in the centre of the endosperm, but white-belly grains have chalkiness in the peripheral part of the endosperm. Considering that starch is actively accumulated around the centre of the endosperm from early to middle stages and at the periphery at the late stage, the differences in the location of chalkiness can thus explain the different response of these two types of chalk to high-temperature stress (Tsukaguchi and Iida 2008) and alteration of source-sink ratio (Cheng et al. 2007). SEM results of the present study demonstrate that white-belly tissues differ with white-core tissue in the arrangement of starch granules and protein bodies, with the later containing no visible protein bodies whereas the former having numbers of protein bodies surrounding starch granules. This observation further suggests the different underlying mechanism between the occurrence of WBRK and WCRK.

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