

# The effects of nitrogen uptake before and after heading on grain protein content and the occurrence of basal- and back-white grains in rice (*Oryza sativa* L.)

Tadashi Tsukaguchi, Yae Taniguchi & Rie Ito

To cite this article: Tadashi Tsukaguchi, Yae Taniguchi & Rie Ito (2016) The effects of nitrogen uptake before and after heading on grain protein content and the occurrence of basal- and back-white grains in rice (*Oryza sativa* L.), *Plant Production Science*, 19:4, 508-517, DOI: [10.1080/1343943X.2016.1223527](https://doi.org/10.1080/1343943X.2016.1223527)

To link to this article: <https://doi.org/10.1080/1343943X.2016.1223527>



© 2016 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group



Published online: 25 Aug 2016.



Submit your article to this journal [↗](#)



Article views: 903



View related articles [↗](#)



Citing articles: 4 View citing articles [↗](#)

## The effects of nitrogen uptake before and after heading on grain protein content and the occurrence of basal- and back-white grains in rice (*Oryza sativa* L.)

Tadashi Tsukaguchi, Yae Taniguchi and Rie Ito

Faculty of Bioresources and Environmental Sciences, Ishikawa Prefectural University, Nonouchi, Japan

### ABSTRACT

Chalky rice (*Oryza sativa* L.) grains are induced by high temperature during the grain-filling period. Plant nitrogen status also affects the occurrence of basal- and back-white grains (*BBWG*). The objective of this study was to elucidate the relations between nitrogen availability per spikelet during the grain-filling period ( $N_{GF}$ ) and each of the percentage of *BBWG* and grain protein content (*GPC*). We further compared the effect of the components of  $N_{GF}$  determined before heading ( $N_{BH}$ ) and after heading ( $N_{AH}$ ) on *BBWG* and *GPC*. We grew the rice cultivar 'Koshihikari' in pots in 2012 and 2013, and top-dressed nitrogen at the panicle formation and heading stages, under two (2012) or three (2013) temperature regimes during the grain-filling period. *GPC* was explained well by  $N_{GF}$  but *BBWG* was not. *BBWG* was best explained in a multiple-regression equation by mean air temperature after heading and by  $N_{BH}$  and  $N_{AH}$ . The partial correlation coefficients for  $N_{BH}$  were 1.6 and 3.0 times those for  $N_{AH}$  in 2012 and 2013, respectively. On the other hand, in a multiple-regression equation for *GPC*, the partial correlation coefficients for  $N_{BH}$  were 0.91 and 0.71 times those for  $N_{AH}$  in 2012 and 2013, respectively. These results suggest that rice grains are most sensitive to plant nitrogen status before heading for *BBWG* but after heading for *GPC*, and that there is an optimal timing for nitrogen top-dressing that would maximize the reduction in *BBWG* per unit increment of *GPC*.

**Abbreviations:** *BBWG*, grain with chalkiness in either the basal or dorsal (back) part of the endosperm ('basal- and back-white grain'); DAH, days after heading; *GPC*, grain protein content; *MWG*, milky-white grain;  $N_{AH}$ , the amount of nitrogen uptake per spikelet after heading;  $N_{BH}$ , the amount of nitrogen uptake per spikelet before heading and that can be remobilized to the panicles;  $N_{GF}$ , the amount of nitrogen per spikelet available for developing grains

### ARTICLE HISTORY

Received 4 June 2016  
Revised 5 August 2016  
Accepted 8 August 2016

### KEYWORDS

Basal-white grain; chalky grain; grain protein content; plant nitrogen status; quality; rice; white-back grain

### CLASSIFICATION

Crop Physiology

High temperature during the grain-filling period decreases rice (*Oryza sativa* L.) grain quality by increasing the proportion of chalky grains. Chalky grains are also induced by a number of plant factors. There are several types of chalky grains, and the type depends on the chalky portion of the endosperm, which is most affected by temperatures during a specific high-temperature-sensitive stage (Tashiro & Wardlaw, 1991). The occurrence of chalky grains can be reduced by improvement of plant nitrogen status (Takata et al., 2010; Tanaka et al., 2010; Wakamatsu et al., 2008). Top-dressing with nitrogen at the heading stage reduced the occurrence of milky-white grains (*MWG*), back-white grains, and basal-white grains, whereas the effect of nitrogen on reducing the occurrence of *MWG* was through increasing the assimilate supply during the grain-filling period, but not through it on the occurrence of basal-white grains and back-white grains (Nakagawa et al., 2006). The occurrence of

basal-white grains and white-back grains is unaffected by the assimilate supply (Tsukaguchi et al., 2011).

These previous studies suggest that plant nitrogen status directly affects the percentage of basal-white grains and back-white grains, although the mechanism is not known. As basal-white grains and back-white grains are induced by similar factors, and chalkiness often occurs in both the back and basal parts of a single grain (Kobayashi et al., 2007), we defined grains with chalkiness in either part of the endosperm as basal- and back-white grains (*BBWG*) in this study.

Plant nitrogen status during the later growth stages also affects the grain protein content (*GPC*) in rice (Mori et al., 2010; Tsukaguchi et al., 2016). *GPC* affects the texture of cooked rice, with high values increasing the hardness and decreasing the stickiness (Hamaker & Griffin, 1990; Martin & Fitzgerald, 2002). In Japan, where tender sticky cooked rice is favored, high *GPC* therefore decreases the

eating quality of rice (Matsue et al., 2001). This suggests that excessive nitrogen application during later growth stages may decrease eating quality.

Thus, efforts to improve the appearance and eating quality of rice seem to create conflicting requirements for plant nitrogen status. Indeed, a negative correlation between *GPC* and *BBWG* has been reported (Nakagawa et al., 2006; Wakamatsu et al., 2008). Therefore, careful nitrogen management is required to maximize the effect of nitrogen to reduce *BBWG* while keeping *GPC* down. To support this management, it is necessary to elucidate the relations between plant nitrogen status and both *BBWG* and *GPC*.

In this study, we used nitrogen availability per spikelet during the grain-filling stage ( $N_{GF}$ ) to describe the plant's nitrogen status during this period. The total amount of nitrogen available for the grains equals the sum of the new uptake during the grain-filling period and the amount taken up before heading and that can be remobilized from the leaves (Yoshida et al., 2016). Specifically,  $N_{GF}$  is composed of nitrogen uptake before heading ( $N_{BH}$ ) and after heading ( $N_{AH}$ ). The objective of this study was to elucidate the relations between  $N_{GF}$  and *BBWG* and between  $N_{GF}$  and *GPC*. We determined and compared the contributions of  $N_{BH}$  and  $N_{AH}$  to *GPC* and *BBWG*. We performed our experiment under different temperatures and nitrogen application patterns to provide the basis for the development of optimum nitrogen application technique.

## Materials and methods

### Plant materials

Pot experiments were conducted at Ishikawa Prefectural University in Nonoichi, Japan, in 2012 and 2013, with the cultivar 'Koshihikari'. Seeds were sown in paper pots (R-5, Nippon Beet Sugar Manufacturing, Tokyo, Japan). On 1 June 2012 and 31 May 2013, 30-day-old seedlings were transplanted into Wagner pots (16.0 cm in diameter and 19.0 cm in depth), at a density of 2 per pot. The pots were filled with sandy soil that received a basal dressing of 0.6 g of N as ammonium sulfate, 1.0 g ( $P_2O_5$  equivalent) of P as superphosphate, and 1.0 g ( $K_2O$  equivalent) of K as potassium chloride. Plants were grown under outdoor conditions until the start of the experimental temperature treatment in both years.

In 2012, the plants received two levels of nitrogen top-dressing at the panicle formation stage (0 and 0.5 g N per pot; 21 days before heading), three levels at the heading stage (0, 0.2, and 0.5 g N per pot), and two levels of temperature treatment, as described in the next section ( $n = 12$  plots in randomized complete block design). Eight pots were prepared for each plot. Additionally, eight pots for each level of top-dressing at the panicle formation stage were prepared for the measurement at heading.

In 2013, the plants received three levels of nitrogen top-dressing at the panicle formation stage (0, 0.2, and 0.4 g N per pot; 20 days before heading), two levels at heading (0 and 0.3 g N per pot), and three levels of temperature treatment ( $n = 18$  plots). Eight pots were prepared for each plot and for each level of top-dressing at the panicle formation stage for the measurement at heading. Nitrogen top-dressing was applied as ammonium sulfate.

### Temperature treatments

Two days after heading, a temperature treatment was applied using two partitioned temperature-gradient chambers (TGCs; Nakagawa et al., 2009). Each TGC was a greenhouse covered with vinyl sheeting, 21 m in length, 3.6 m in width, and 2.4 m in maximum height. It was partitioned with vinyl sheeting into three rooms, each 7 m in length. We designated the northern, middle, and southern rooms as T1, T2, and T3, respectively. The sides of the T1 room were left open, and two exhaust fans were mounted at the southern end of the T3 room, generating continuous air flow from the T1 to the T3. Air flowed from one room to the next through a vent at the bottom of the partitioning vinyl sheet and three air hoses. The exhaust capacity of one fan could be varied between 0 and  $1.40 \text{ m}^3 \text{ s}^{-1}$  by means of a regulating inverter, whereas the other fan operated at a constant rate of  $0.35 \text{ m}^3 \text{ s}^{-1}$ .

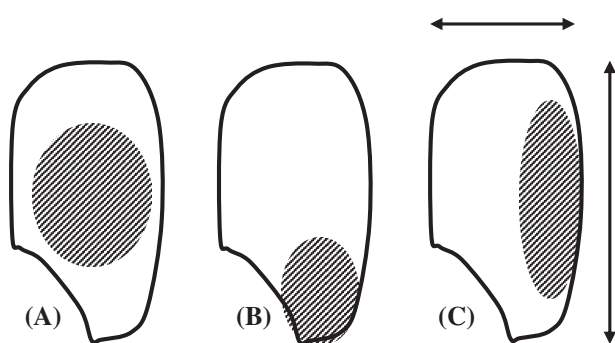
Two oil heaters were placed in the T2 room and another two in the T3 room. The air temperature in the T1 room was close to ambient because of the air flow through the open sides, whereas that of the T3 room was highest owing to the generated air flow, which was heated by solar radiation and the heater. The air temperature of the T3 room was targeted at  $34 \text{ }^\circ\text{C}$  during the day and  $28 \text{ }^\circ\text{C}$  at night, and was maintained near these levels by regulating the fan exhaust rate and the heat supply. The air in each small room was dispersed using two circulators. Table 1 summarizes the temperature conditions in the rooms. The ambient temperature was higher in 2012 than in 2013 during the stages from panicle formation to heading, from heading to 20 days after heading (DAH), and from 21 DAH to maturity. The amount of solar radiation was also higher in 2012 than in 2013 during all three stages. In 2012, mean air temperatures in T2 were  $1.6 \text{ }^\circ\text{C}$  and  $2.5 \text{ }^\circ\text{C}$  higher than those in T1 from heading to 20 DAH and from 21 DAH to maturity. The mean air temperature in the TGC ranged from  $27.4$  to  $30.7 \text{ }^\circ\text{C}$  from heading to 20 DAH and from  $24.5$  to  $28.8 \text{ }^\circ\text{C}$  from 21 DAH to maturity in 2013.

Each room housed a water bed 3.7 m in length and 1.7 m in width, in which we placed the pots. The water level was kept from 2 to 5 cm above the top of the pots throughout the treatment. Half of the pots of each plot were randomly arranged in each of the two TGCs. In 2012,

**Table 1.** Meteorological data.

	Mean air temperature (°C)			Solar radiation (MJ m <sup>-2</sup> )		
	20 DBH <sup>a</sup> -H <sup>b</sup>	H-20 DAH <sup>c</sup>	21 DAH-M <sup>d</sup>	20 DBH-H	H-20 DAH	21 DAH-M
2012						
Ambient	27.8	28.3	26.2	346.4	305.9	285.0
T1		28.2	26.9			
T2		29.9	29.4			
2013						
Ambient	26.5	27.3	23.7	282.5	291.8	193.6
T1		27.4	24.5			
T2		29.4	27.2			
T3		30.7	28.8			

<sup>a</sup>DBH: days before heading; <sup>b</sup>H: heading; <sup>c</sup>DAH: days after heading; <sup>d</sup>M: maturity.



**Figure 1.** Schematic picture of milky-white grain (A), basal-white grain (B) and back-white grain (C). Milky-white grains were those with central chalkiness (shaded area) that covered more than half of the endosperm; basal-white grains were those with basal chalkiness that accounted for >20% of the endosperm; and back-white grains were those with dorsal chalkiness that covered >2/3 of the length (vertical arrow and >1/3 of the width horizontal arrow).

because of the high ambient temperature and the high amount of solar radiation during the day, the fan exhaust rate continued to be high causing strong wind in the TGC, so most of the grains became opaque and could not be classified into different types of chalky grains in room T3, possibly owing to the combined effect of the high temperature and strong wind (Morita et al., 2016). Therefore, we did not use the data from the T3 room in 2012 in our analysis.

### 3. Measurement of dry weight and nitrogen content

Plants were sampled at the heading stage and at maturity. Maturity was defined as the date on which more than 95% of the spikelets became yellow. Plants of eight pots harvested at heading were separated into the leaf blade, leaf sheath+culm, panicle, and dead parts, and were then dried for 72 h at 80 °C and weighed. Plants harvested at maturity were separated into the same parts, and all parts except the panicles were dried for 72 h at 80 °C and weighed. The panicles were air-dried for 1 week, and then divided into

two subsamples, which were each weighed. One of the subsamples was then dried for 72 h at 80 °C and used to determine the moisture content of the air-dried panicles and the nitrogen content. The other subsample was used to determine grain yield and quality.

Each oven-dried sample was ground into a powder fine enough to pass through a 0.5-mm screen using a CSM-F1 Cyclone Sample Mill (Udy Corp., Ft. Collins, CO, USA). The nitrogen content was measured using the Dumas combustion method with an NCH Analyzer (Sumika Chemical Analysis Service, Tokyo, Japan).

### Measurement of yield and grain quality

Panicles in the second subsample were threshed, and the spikelets were counted. Spikelets were then hulled, and the number, the weight and the moisture content of total grains thicker than 1.6 mm were determined. Single-grain weight was calculated as the mean grain weight and adjusted to 14% moisture content. The percentages of milky-white grains, basal-white grains, and back-white grains were determined visually (Figure 1). Milky-white grains (MWG) were defined as those with central chalkiness that covered more than half of the endosperm; basal-white grains were defined as those with basal chalkiness that accounted for >20% of the endosperm; and back-white grains were defined as those with dorsal chalkiness that covered >2/3 of the length and >1/3 of the width. Grains with chalkiness in more than two parts of the endosperm were included in the count for all the types of chalkiness (i.e. were counted twice or three times). BBWG were grains either with basal chalkiness or dorsal chalkiness.

The grain from all categories was then combined and ground into a powder, and nitrogen content was measured with the NCH analyzer. The protein content was then calculated by multiplying the nitrogen content by 5.95 (Jones, 1941). The moisture content of each sample was determined and GPC was adjusted to 14% grain moisture content.

### Parameters for plant nitrogen status

Nitrogen availability per spikelet during the grain-filling period ( $N_{GF}$ : mg per spikelet) was calculated as the sum of the amount of nitrogen uptake before heading (expressed per spikelet) that was available to be remobilized to developing grains ( $N_{BH}$ ) and the amount of nitrogen uptake per spikelet after heading ( $N_{AH}$ ):

$$N_{GF} = N_{BH} + N_{AH}$$

$N_{BH}$  and  $N_{AH}$  were calculated as:

$$N_{BH} = \frac{NV_H - 0.005WV_H}{\text{Number of spikelets}} \times 10^3$$

$$N_{AH} = \frac{\Delta N_{HtoM}}{\text{Number of spikelets}} \times 10^3$$

where  $NV_H$  and  $WV_H$  represent the nitrogen content (g per plant) and dry weight of vegetative parts at heading, respectively, and  $\Delta N_{HtoM}$  represents the nitrogen uptake during the period from heading to maturity (g per plant). The constant 0.005 represents the nitrogen content remaining in dead parts.

### Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS v. 21 (SPSS Inc., Chicago, IL, USA) to assess the effects of mean

air temperature during the 20 days after heading, effects of nitrogen top-dressing at the panicle formation stage, and effects of nitrogen top-dressing at heading. When the ANOVA result was significant, significant differences between pairs of mean values were detected using Tukey's test. Significance was defined as  $p < 0.05$ , unless otherwise noted. Multiple-regression analysis was conducted to determine the relative contributions of  $N_{BH}$  and  $N_{AH}$  to  $BBWG$ ,  $MWG$  and  $GPC$ . We tested for homogeneity of the regression coefficients between  $GPC$  and  $N_{GF}$  according to the method of Gomez and Gomez (1976).

### Results

Top-dressing at the panicle formation stage reduced  $BBWG$  (averaged over the temperature and top-dressing at heading treatments) from 76 to 59% in 2012 and from 65 to 24% in 2013 (Tables 2 and 3). Top-dressing at heading reduced  $BBWG$  (averaged over the temperature and top-dressing at panicle formation treatments) from 73 to 60% in 2012 and from 48 to 39% in 2013. Whereas top-dressing at panicle formation had no significant effect on  $MWG$  in either year, top-dressing at heading reduced it, significantly from 24 to 13% in 2012 and from 25 to 20% in 2013 (not significantly).  $GPC$  was not significantly affected by temperature in either year. Both top-dressings significantly increased  $GPC$ . Top-dressing at the panicle formation stage increased

**Table 2.** Percentages of basal- and back-white grains ( $BBWG$ ) and milky-white grains ( $MWG$ ), grain protein content ( $GPC$ ), number of spikelets, and single-grain weight in 2012.

T <sup>a</sup>	PF	H	$BBWG$ (%)	$MWG$ (%)	$GPC$ (%)	Number of spikelet	Single-grain weight (mg)
T1	0	0	71.1	8.8	5.9	1268	19.1
T2	0	0	84.8	40.1	5.9	1273	18.0
T1	0.5	0	55.3	8.9	7.0	1539	21.5
T2	0.5	0	81.0	37.2	7.4	1500	20.3
T1	0	0.2	66.6	3.6	6.9	1238	19.1
T2	0	0.2	81.7	20.4	7.1	1129	18.2
T1	0.5	0.2	42.0	5.3	7.7	1653	21.6
T2	0.5	0.2	77.8	34.5	7.8	1540	20.7
T1	0	0.5	63.6	5.2	7.8	1217	20.2
T2	0	0.5	78.2	20.8	8.0	1284	19.3
T1	0.5	0.5	30.1	3.8	8.1	1575	21.9
T2	0.5	0.5	66.5	22.8	8.4	1571	21.1
Results of ANOVA							
T1			54.8	5.9	7.2	1415	20.6
T2			79.5	29.3	7.4	1383	19.6
T			***	***	ns	ns	**
0			75.5	16.5	6.9	1235	19.0
0.5			58.8	18.8	7.7	1563	21.2
N at PF <sup>b</sup>			**	ns	***	***	**
0			73.1b	23.8a	6.5a	1395	19.7
0.2			68.3b	16.0b	7.4b	1390	19.9
0.5			60.1a	13.1b	8.1c	1412	20.6
N at H <sup>c</sup>			*	*	***	ns	ns
T × N at PF			ns	ns	ns	ns	ns
T × N at H			ns	ns	ns	ns	ns
N at PF × N at H			ns	ns	*	ns	ns

<sup>a</sup>T, temperature regime; <sup>b</sup>N at PF, nitrogen top-dressing at panicle formation stage; <sup>c</sup>N at H, nitrogen top-dressing at heading.

\*, \*\*, and \*\*\*, significant at the 0.05, 0.01, and 0.001 level, respectively; ns, not significant by ANOVA. Values with the same letter are not significantly different at the 0.05 probability level by Tukey's test.

GPC from 6.9 to 7.7% in 2012 and from 6.9 to 8.2% in 2013. Top-dressing at heading increased GPC from 6.5 to 8.1% in 2012 and from 7.0 to 8.2% in 2013. Interaction between top-dressing at the panicle formation stage and that at heading was significant ( $p < 0.05$  in both years).

Top-dressing at panicle formation significantly increased the number of spikelets: from 1235 to 1563 in 2012 and from 1109 to 1541 in 2013 (Tables 2 and 3). High temperature significantly decreased the single-grain weight in both years (Tables 2 and 3). In both years, top-dressing significantly increased the single-grain weight at the panicle formation stage, but there was no effect of top-dressing at heading.

Temperature had no significant effect on dry weight, nitrogen uptake, or nitrogen content at maturity in either year (Tables 4 and 5). However, both top-dressings markedly and significantly increased all three parameters.

There was generally no significant correlation between  $BBWG$  and  $N_{GF}$  except for a significant negative correlation in T2 in 2012 (Figure 2). There was no significant correlation between  $MWG$  and  $N_{GF}$  (Figure 3). There was a significant positive logarithmic relation between  $N_{GF}$  and GPC in both

years (Figure 4). The homogeneity test of the regression coefficients revealed that there was no significant difference in the  $N_{GF}$ -GPC relation between temperature conditions or between years.

We used multiple-regression analysis to determine the relative contributions of  $N_{AH}$  and  $N_{BH}$  to  $BBWG$ ,  $MWG$  and GPC (Table 6). With the mean air temperature during the 20 DAH ( $T$ ),  $N_{AH}$  and  $N_{BH}$  as independent variables, the overall regression for  $BBWG$  was highly significant:  $R^2 = 0.889$  in 2012 ( $p < 0.001$ ,  $n = 12$ ) and  $R^2 = 0.976$  in 2013 ( $p < 0.001$ ,  $n = 18$ ). Regression coefficients for  $T$ ,  $N_{AH}$  and  $N_{BH}$  were all significant in both years. Partial correlation coefficients for  $N_{BH}$  were about 1.6 times (2012) and 3.0 times (2013) those for  $N_{AH}$  (Table 6). The overall regression for  $MWG$  (Table 7) was highly significant, with  $R^2 = 0.891$  in 2012 ( $p < 0.001$ ,  $n = 12$ ) and  $R^2 = 0.765$  in 2013 ( $p < 0.001$ ,  $n = 18$ ). Regression coefficients for  $T$  were significant in both years, but those for  $N_{AH}$  in 2012 and  $N_{BH}$  in either year were not significant. The overall regression for GPC (Table 8) was highly significant, with  $R^2 = 0.893$  in 2012 ( $p < 0.001$ ,  $n = 12$ ) and  $R^2 = 0.876$  in 2013 ( $p < 0.001$ ,  $n = 18$ ). Regression coefficients for  $T$  were not significant in either year, but those for

**Table 3.** Percentages of basal- and back-white grains ( $BBWG$ ) and milky-white grains ( $MWG$ ), grain protein content (GPC), number of spikelets, and single-grain weight in 2013.

T <sup>a</sup>	PF	H	$BBWG$ (%)	$MWG$ (%)	GPC (%)	Number of spikelet	Single-grain weight (mg)
T1	0	0	50.2	6.1	6.1	1116	18.6
T2	0	0	78.5	30.6	6.0	1104	17.9
T3	0	0	86.1	55.3	6.1	1131	16.0
T1	0	0.3	33.1	9.3	7.8	1094	19.4
T2	0	0.3	64.1	9.6	7.5	1133	18.7
T3	0	0.3	78.2	25.0	8.1	1078	17.6
T1	0.2	0	27.7	5.5	6.9	1421	20.4
T2	0.2	0	48.5	16.5	7.0	1347	19.7
T3	0.2	0	64.2	45.2	7.2	1390	17.7
T1	0.2	0.3	18.7	7.5	8.3	1470	19.5
T2	0.2	0.3	34.8	18.0	8.7	1401	19.1
T3	0.2	0.3	57.7	42.5	8.3	1495	18.1
T1	0.4	0	7.2	8.0	8.0	1576	19.6
T2	0.4	0	25.1	16.6	8.0	1585	19.5
T3	0.4	0	43.8	36.5	7.8	1488	18.7
T1	0.4	0.3	4.6	8.3	8.6	1523	21.0
T2	0.4	0.3	25.2	20.2	8.5	1546	19.1
T3	0.4	0.3	38.2	35.4	8.2	1529	18.3
Results of ANOVA							
T1			23.6c	7.4c	7.6	1367	19.8
T2			46.0b	18.6b	7.6	1353	19.0
T3			61.3a	40.0a	7.6	1352	17.7
T			***	***	ns	ns	***
0			65.0a	22.7	6.9c	1109c	18.0
0.2			41.9b	22.5	7.7b	1421b	19.1
0.4			24.0c	20.8	8.2a	1541a	19.4
N at PF <sup>b</sup>			***	ns	***	***	*
0			47.9	24.5	7.0	1351	18.7
0.3			39.4	19.5	8.2	1363	19.0
N at H <sup>c</sup>			*	ns	***	ns	ns
T × N at PF			ns	ns	ns	ns	ns
T × N at H			ns	ns	ns	ns	ns
N at PF × N at H			ns	ns	*	ns	ns

<sup>a</sup>T, temperature regime; <sup>b</sup>N at PF, nitrogen top-dressing at panicle formation stage; <sup>c</sup>N at H, nitrogen top-dressing at heading.

\*, \*\*, and \*\*\*, significant at the 0.05, 0.01, and 0.001 level, respectively; ns, not significant by ANOVA. Values with the same letter are not significantly different at the 0.05 probability level by Tukey's test.

**Table 4.** Aboveground dry weight at maturity (*DW*), nitrogen uptake at maturity, nitrogen content at maturity, and dry weight per spikelet at maturity (*DW* per spikelet) in 2012.

T <sup>a</sup>	PF	H	<i>DW</i> (g)	Nitrogen uptake (g)	Nitrogen content (%)	<i>DW</i> per spikelet (mg spikelet <sup>-1</sup> )
T1	0	0	65.8	0.44	0.67	51.9
T2	0	0	63.4	0.43	0.68	49.8
T1	0.5	0	85.6	0.60	0.70	55.6
T2	0.5	0	84.5	0.60	0.71	56.3
T1	0	0.2	73.8	0.52	0.70	59.6
T2	0	0.2	71.5	0.48	0.68	63.3
T1	0.5	0.2	98.4	0.79	0.80	59.5
T2	0.5	0.2	93.4	0.75	0.80	60.6
T1	0	0.5	79.2	0.67	0.85	65.1
T2	0	0.5	80.4	0.69	0.85	62.6
T1	0.5	0.5	100.1	0.95	0.95	63.5
T2	0.5	0.5	102.4	0.97	0.96	65.2
Results of ANOVA						
T1			83.8	0.66	0.78	59.2
T2			82.6	0.65	0.78	59.6
T			ns	ns	ns	ns
0			72.4	0.54	0.74	58.7
0.5			94.0	0.78	0.82	60.1
N at PF <sup>b</sup>			***	***	***	ns
0			74.8c	0.52c	0.69c	53.4c
0.2			84.3b	0.64b	0.75b	60.8b
0.5			90.5a	0.82a	0.90a	64.1a
N at H <sup>c</sup>			***	***	***	***
T × N at PF			ns	ns	ns	ns
T × N at H			ns	ns	ns	ns
N at PF × N at H			ns	ns	ns	ns

<sup>a</sup>T, temperature regime; <sup>b</sup>N at PF, nitrogen top-dressing at panicle formation stage; <sup>c</sup>N at H, nitrogen top-dressing at heading.

\*, \*\*, and \*\*\*, significant at the 0.05, 0.01, and 0.001 level, respectively; ns, not significant by ANOVA. Values with the same letter are not significantly different at the 0.05 probability level by Tukey's test.

$N_{AH}$  and  $N_{BH}$  were significant in both years. Partial correlation coefficients for  $N_{BH}$  were 0.91 times (2012) and 0.71 times (2013) those for  $N_{AH}$  (Table 8), respectively.

## Discussion

Nitrogen top-dressing at the panicle formation stage and at heading significantly reduced *BBWG* in both years (Tables 2 and 3). On the other hand, only that at heading reduced *MWG* significantly in 2012 and not significantly in 2013. The different responses of *BBWG* and *MWG* to nitrogen top-dressings can be partially explained by the direct effect of plant nitrogen status on *BBWG* and its indirect effect on *MWG* through its effect on dry matter production (Nakagawa et al., 2006; Yoshida et al., 2016). Carbohydrate availability explains *MWG* but not basal-white or back-white grains (Tsukaguchi et al., 2011). Both top-dressings increased dry matter production, but that at the panicle formation stage also increased the number of spikelets; thus, the dry matter production per spikelet did not increase significantly (Tables 4 and 5), suggesting carbohydrates availability may not be increased. Under the temperature conditions in T2 and T3 (Table 1), a considerable percentage of *MWG* may have been those with chalkiness at the center of the endosperm cross-section, which occurs irrespective of the assimilate supply (Tsukaguchi

et al., 2012). Both top-dressings reduced *BBWG*, regardless of the assimilate supply per spikelet, which agrees well with previous studies (Nakagawa et al., 2006).

We found a significant positive logarithmic relation between  $N_{GF}$  and *GPC* (Figure 4), which agreed with the results of Tsukaguchi et al. (2016), who found a cultivar-specific logarithmic relation between nitrogen availability per unit sink capacity during the grain-filling period and *GPC*. In the present study, we used the number of spikelets instead of sink size because the potential grain size is largely determined genetically (Matsushima, 1995). Multiple-regression analysis revealed a stronger effect of  $N_{AH}$  than  $N_{BH}$  on *GPC* (Table 8). Therefore, a more accurate relation between *GPC* and plant nitrogen status would be obtained by accounting for the different contributions of  $N_{BH}$  and  $N_{AH}$  to *GPC*. Nevertheless, *GPC* was strongly logarithmically related to  $N_{GF}$ ; thus,  $N_{GF}$  can be regarded as an approximate measure of the plant's nitrogen status and is responsible for *GPC*. There were no significant effect of temperature on *GPC* (Table 8) and no significant difference among temperature conditions in the regression coefficients in either year. Therefore, *GPC* is explained well by  $N_{GF}$  irrespective of the timing of nitrogen application and temperature conditions.

*BBWG* was not significantly correlated with  $N_{GF}$  except for a significant negative correlation at T2 in 2012 (Figure 2).

**Table 5.** Aboveground dry weight at maturity (*DW*), nitrogen uptake at maturity, nitrogen content at maturity, and dry weight per spikelet at maturity (*DW* per spikelet) in 2013.

T <sup>a</sup>	PF	H	<i>DW</i> (g)	Nitrogen uptake (g)	Nitrogen content (%)	<i>DW</i> per spikelet (mg spikelet <sup>-1</sup> )
T1	0	0	61.2	0.43	0.71	54.9
T2	0	0	57.4	0.39	0.67	52.0
T3	0	0	58.1	0.39	0.67	51.4
T1	0	0.3	66.8	0.63	0.95	61.1
T2	0	0.3	67.8	0.64	0.95	59.8
T3	0	0.3	67.0	0.65	0.98	62.2
T1	0.2	0	72.1	0.62	0.86	50.7
T2	0.2	0	68.1	0.57	0.84	50.5
T3	0.2	0	64.9	0.54	0.84	46.7
T1	0.2	0.3	78.4	0.86	1.10	53.4
T2	0.2	0.3	80.9	0.86	1.08	57.7
T3	0.2	0.3	80.9	0.84	1.05	54.1
T1	0.4	0	76.9	0.75	0.98	48.8
T2	0.4	0	79.5	0.74	0.94	50.1
T3	0.4	0	79.2	0.76	0.97	53.2
T1	0.4	0.3	90.3	0.99	1.10	59.3
T2	0.4	0.3	96.4	1.03	1.07	62.3
T3	0.4	0.3	96.7	1.03	1.07	63.3
Results of ANOVA						
T1			74.3	0.71	0.95	54.7
T2			75.0	0.71	0.92	55.4
T3			74.5	0.70	0.93	55.1
T			ns	ns	ns	ns
0			63.1c	0.52c	0.82c	56.9
0.2			74.2b	0.72b	0.96b	52.2
0.4			86.5a	0.88a	1.02a	56.2
N at PF <sup>b</sup>			***	***	***	ns
0			68.6	0.58	0.83	50.9
0.3			80.6	0.84	1.04	59.2
N at H <sup>c</sup>			***	***	***	***
T × N at PF			ns	ns	ns	ns
T × N at H			ns	ns	ns	ns
N at PF × N at H			ns	ns	ns	ns

<sup>a</sup>T, temperature regime; <sup>b</sup>N at PF, nitrogen top-dressing at panicle formation stage; <sup>c</sup>N at H, nitrogen top-dressing at heading.

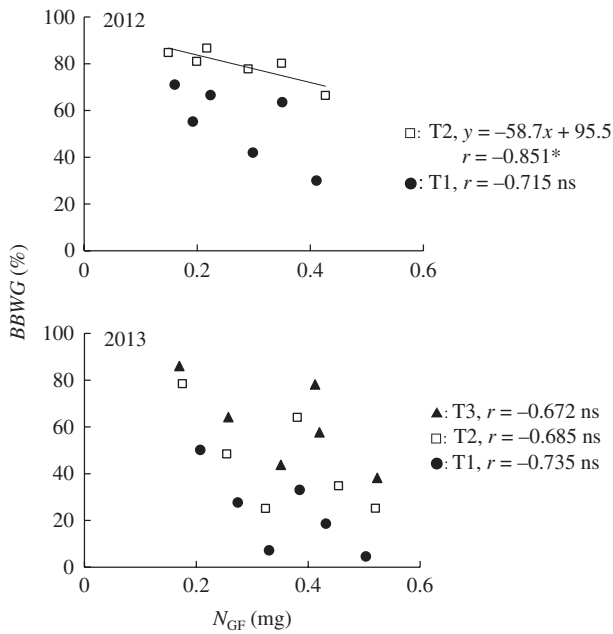
\*, \*\*, and \*\*\*, significant at the 0.05, 0.01, and 0.001 level, respectively; ns, not significant by ANOVA. Values with the same letter are not significantly different at the 0.05 probability level by Tukey's test.

The multiple regression analysis revealed a larger contribution of  $N_{BH}$  than of  $N_{AH}$  to reducing *BBWG* (Table 6). The increase in  $N_{GF}$  due to the top-dressing at heading had a smaller effect than that due to top-dressing at the panicle formation stage on reducing *BBWG*. Therefore, the responsibility of plant nitrogen status for the occurrence of *BBWG* should be evaluated from the different effects of  $N_{BH}$  and  $N_{AH}$ . We found no correlation between  $N_{BH}$  and  $N_{AH}$  owing to the different nitrogen top-dressings at the panicle formation stage and at heading, which suggests that  $N_{GF}$  is not an appropriate measure for the effect of plant nitrogen status on *BBWG*. However, Yoshida et al. (2016) showed a close relation between the percentage of chalky grains or *BBWG* and nitrogen availability per spikelet using a crop growth model. The data used in their model were obtained from plants with various amounts and patterns of nitrogen application as basal- and top-dressings at 20–31 days after transplanting, 15–16 days before heading, and 7–9 days before heading. In their study, there may have been a positive correlation between  $N_{BH}$  and  $N_{AH}$  because there was no top-dressing after 7–9 days before

heading. Unless a top-dressing at heading is applied,  $N_{GF}$  might be an approximate measure of the effect of plant nitrogen status on *BBWG*, but further study will be required to confirm this hypothesis.

*GPC* and *BBWG* responded differently to  $N_{BH}$  and  $N_{AH}$  and thus to  $N_{GF}$  and our results suggest that plant nitrogen status most strongly affects *BBWG* earlier than *GPC*. Consequently, top-dressing at heading has less effect on reducing *BBWG* per unit increment of *GPC*. Nakagawa et al. (2012) proposed a model that predicted *BBWG* from three explanatory variables (*GPC*, air temperature and solar radiation during 20 days after heading) from field data obtained in 2010 over a wide region, from Tohoku to Hokuriku, and estimated that the effect on *BBWG* of a 1% decrease in *GPC* was equivalent to that of a 2.4 °C rise in air temperature during the 20 days after heading. However, our results suggest that the effect of increased *GPC* would be lower if it is caused by top-dressing at heading. The application of top-dressing at heading has been uncommon because it decreases the eating quality by increasing *GPC* (Terashima et al., 2001). In addition to the

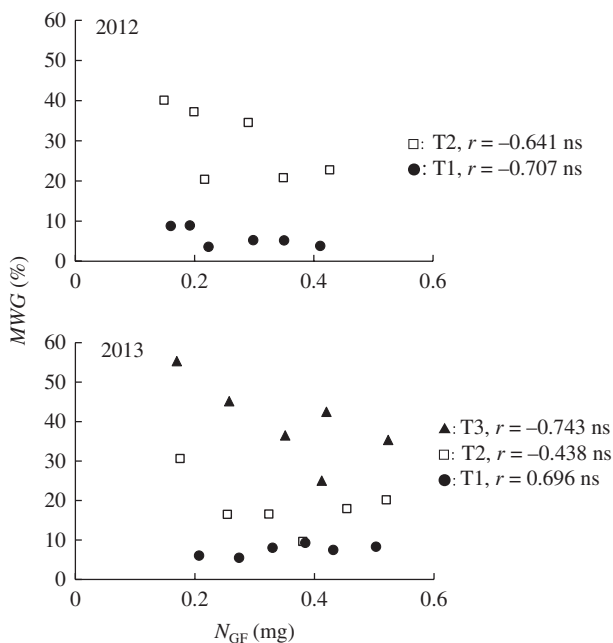




**Figure 2.** Relation between nitrogen availability per spikelet ( $N_{GF}$ ) and percentage of basal-white and white-back grains ( $BBWG$ ).

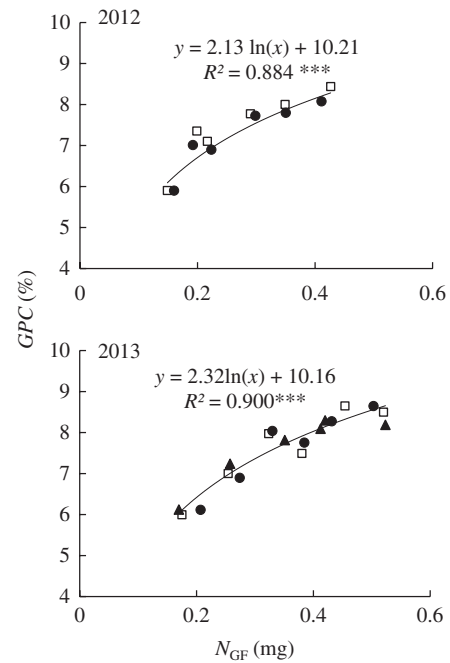
Notes. Solid circles, T1; open squares, T2; solid triangles, T3. \*, significant at the 0.05 level, ns, not significant.

effect on  $GPC$ , the lower ability to reduce  $BBWG$  per unit increment of  $GPC$  may have been recognized. Our results strongly suggest that there is an optimal timing for nitrogen top-dressing that will maximize the reduction in  $BBWG$  per unit increment of  $GPC$ . However, additional research will be required to identify that timing.



**Figure 3.** Relation between nitrogen availability per spikelet ( $N_{GF}$ ) and percentage of milky-white grains ( $MWG$ ).

Notes. Solid circles, T1; open squares, T2; solid triangles, T3. ns, not significant.



**Figure 4.** Relation between nitrogen availability per spikelet ( $N_{GF}$ ) and grain protein content ( $GPC$ ).

Notes. Solid circles, T1; open squares, T2; solid triangles, T3. \*\*\*, significant at the 0.001 level.

The high relative importance of nitrogen uptake before heading for the reduction of  $BBWG$  suggests that  $BBWG$  is most susceptible to plant nitrogen status before heading. Although it is important to identify the critical stage responsible for  $BBWG$ , it would be difficult to identify it precisely and to evaluate the effect of nitrogen availability at this critical stage on the reduction of  $BBWG$ . Nitrogen status before heading affects many interrelated traits associated with grain yield and quality, such as the number of spikelets, leaf area, leaf nitrogen status, and the non-structural carbohydrate content (Batten et al., 1993; Wada, 1981). Therefore, an analysis such as the dynamic modeling performed by Yoshida et al. (2016) will be necessary to evaluate the effect of nitrogen availability at the critical stage and to estimate the nitrogen requirement to optimize grain yield, appearance quality, and eating quality.

Plant nitrogen status may affect grain quality in several ways. Maturation of storage proteins generates  $H_2O_2$  as a byproduct, which is not only one of the toxic reactive oxygen species (ROS) but an important signaling molecule in the regulation of a variety of processes including heat tolerance (Mitsui et al., 2016). Improving plant nitrogen status lowers the canopy temperature through increased transpiration (Xiong et al., 2015); however, how the plant's nitrogen status before heading affects  $BBWG$  is unclear. Nitrogen application at different stages might have different effects on the transpiration rate. However, in the present study, pots in the same nitrogen treatment were not placed in a group,

**Table 6.** Multiple regression analysis with the percentage of *BBWG* as a dependent variable and *T*,  $N_{BH}$ , and  $N_{AH}$  as independent variables.

	F-value	$R^2$	Regression coefficient				Partial correlation coefficient		
			Intercept	<i>T</i>	$N_{BH}$	$N_{AH}$	<i>T</i>	$N_{BH}$	$N_{AH}$
2012	21.4***	0.889	-347.4	15.8***	-177.8**	-64.4*	0.757	-0.578	-0.354
2013	191.5***	0.976	-126.8	8.9***	-349.8***	-55.6***	0.687	-0.693	-0.234

\*, \*\*, \*\*\*, significant at the 0.05, 0.01, 0.001 level, respectively.  
 $n = 12$  in 2012;  $n = 18$  in 2013.

**Table 7.** Multiple regression analysis with the percentage of *MWG* as a dependent variable and *T*,  $N_{BH}$ , and  $N_{AH}$  as independent variables.

	F-value	$R^2$	Regression coefficient				Partial correlation coefficient		
			Intercept	<i>T</i>	$N_{BH}$	$N_{AH}$	<i>T</i>	$N_{BH}$	$N_{AH}$
2012	21.7***	0.891	-395.6	14.4***	-1.70	45.0*	0.878	-0.058	-0.315
2013	15.2***	0.765	-171.0	7.0***	-10.9	-31.4	0.846	-0.034	-0.207

\*, \*\*, \*\*\*, significant at the 0.05, 0.001 level, respectively.  
 $n = 12$  in 2012;  $n = 18$  in 2013.

**Table 8.** Multiple regression analysis with *GPC* as a dependent variable and *T*,  $N_{BH}$ , and  $N_{AH}$  as independent variables.

	F-value	$R^2$	Regression coefficient				Partial correlation coefficient		
			Intercept	<i>T</i>	$N_{BH}$	$N_{AH}$	<i>T</i>	$N_{BH}$	$N_{AH}$
2012	22.3***	0.893	1.20	0.1	10.7***	6.9***	0.122	0.746	0.819
2013	32.9***	0.876	4.7	-0.0	9.8***	6.5***	-0.009	0.538	0.753

\*\*\*, significant at the 0.001 level.  
 $n = 12$  in 2012;  $n = 18$  in 2013.

and were instead placed randomly, and air in the TGCs was circulated constantly. Therefore, it is unlikely that the stronger effect of  $N_{BH}$  than of  $N_{AH}$  on *BBWG* resulted from a higher evaporative cooling capacity. The rice plant's translocation system may affect the occurrence of *BBWG*. Tanaka et al. (2009) observed earlier degradation of the nucellar epidermis under high temperatures in a heat-sensitive cultivar. Nitrogen application at the panicle formation stage increased the cross-sectional area of large vascular bundles (Nitta et al., 2000). Thus, high nitrogen availability may contribute to the development of a robust translocation system that is less susceptible to high temperatures.

In conclusion, *GPC* was explained well by  $N_{GF}$  whereas *BBWG* was not. *BBWG* was highly significantly explained by multiple regression analysis with mean air temperature after heading,  $N_{BH}$ , and  $N_{AH}$  as the independent variables, and the partial correlation coefficients for  $N_{BH}$  were 1.6 times (2012) and 3.0 times (2013) those for  $N_{AH}$ . On the other hand, in the multiple regression analysis for *GPC*, the partial correlation coefficients for  $N_{BH}$  were 0.91 times (2012) and 0.71 times (2013) those for  $N_{AH}$ . These results suggest that *BBWG* is most susceptible to the plant's nitrogen status earlier than *GPC*, and that there would be an optimal timing for nitrogen top-dressing that would maximize the reduction in *BBWG* per unit increment of *GPC*.

## Acknowledgments

We thank Ayumi Sakuragawa of Ishikawa Prefectural University for her assistance in carrying out the experiments.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This study was partially supported by the Research Program on Climate Change Adaptation of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

## References

- Batten, G. D., Blakeney, A. B., McGrath, V. B., & Ciavarella, S. (1993). Non-structural carbohydrate: Analysis by near infrared reflectance spectroscopy and its importance as an indicator of plant growth. *Plant and Soil*, 155–156, 243–246.
- Gomez, K. A., & Gomez, A. A. (1976). *Statistical procedures for agricultural research* (2nd ed.). New York, NY: John Wiley & Sons.
- Hamaker, B. R., & Griffin, V. K. (1990). Changing the viscoelastic properties of cooked rice through protein disruption. *Cereal Chemistry*, 67, 261–264.
- Jones, D. B. (1941). *Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins*. Circular No. 183. Washington, DC: United States Department of Agriculture.
- Kobayashi, A., Genliang, B., Shenghai, Y., & Tomita, K. (2007). Detection of quantitative trait loci for white-back and basal-white kernels under high temperature stress in Japonica rice varieties. *Breeding Science*, 57, 107–116.
- Martin, M., & Fitzgerald, M. A. (2002). Proteins in rice grains influence cooking properties! *Journal of Cereal Science*, 36, 285–294.
- Matsue, Y., Sato, H., & Uchimura, Y. (2001). The palatability and physicochemical properties of milled rice for each grain-thickness group. *Plant Production Science*, 4, 71–76.

- Matsushima, S. (1995). Physiology of high-yielding rice plants from the viewpoint of yield components. In T. Matsuo & K. Hoshikawa (Eds.), *Science of the rice plant* (pp. 737–766). Volume 3. Tokyo: Physiology. Food and Agriculture Policy Research Center.
- Mitsui, T., Yamakawa, H., & Kobata, T. (2016). Molecular physiological aspects of chalking mechanism in rice grains under high-temperature stress. *Plant Production Science*, 19, 22–29.
- Mori, S., Yokoyama, K., & Fujii, H. (2010). Classification of brown rice with different protein content using the diagnosis of leaf color during the ripening period in Shonai area of Yamagata prefecture. *Japanese Journal of Crop Science*, 79, 113–119\*.
- Morita, S., Wada, H., & Matsue, Y. (2016). Countermeasures for heat damage in rice grain quality under climate change. *Plant Production Science*, 19, 1–11.
- Nakagawa, H., Shirakawa, M., & Nagahata, H. (2006). Effects of potential assimilate supply and nitrogen top-dressing on the occurrence of chalky grains in rice. *Japanese Journal of Crop Science*, 75(extra issue 2), 12–13\*\*.
- Nakagawa, H., Tsukaguchi, T., Yamada, H., & Yamamura, T. (2009). Development of compartmented temperature gradient chamber. *Japanese Journal of Crop Science*, 78(extra issue 1), 180–181\*\*.
- Nakagawa, H., Yoshida, H., Ohno, H., Nakazono, K., Kondo, M., Iwasawa, N., ... Inoue, K. (2012). What deteriorated the appearance of Koshihikari rice produced in 2010? *Japanese Journal of Crop Science*, 81(extra issue 1), 126–127\*\*.
- Nitta, Y., Yao, Y., Yamamoto, Y., Yoshida, T., & Matsuda, T. (2000). Varietal differences in the number and cross area of large vascular bundles at the neck internode of rice. *Japanese Journal of Crop Science*, 69, 61–68\*.
- Takata, S., Sakata, M., Kameshima, M., Yamamoto, Y., & Miyazaki, A. (2010). Varietal difference in the relation between the occurrence of white immature kernels caused by a high temperature during the ripening period and the amount of basal nitrogen application in rice. *Japanese Journal of Crop Science*, 79, 150–157\*.
- Tanaka, K., Miyazaki, M., Uchikawa, O., & Araki, M. (2010). Effects of the nitrogen nutrient condition and nitrogen application on kernel quality of rice. *Japanese Journal of Crop Science*, 79, 450–459\*.
- Tanaka, K., Onishi, R., Miyazaki, M., Ishibashi, Y., Yuasa, T., & Iwaya-Inoue, M. (2009). Changes in NMR relaxation of rice grains, kernel quality and physicochemical properties in response to a high temperature after flowering in heat-tolerant and heat-sensitive rice cultivars. *Plant Production Science*, 12, 185–192.
- Tashiro, T., & Wardlaw, I. F. (1991). The effect of high temperature on kernel dimensions and the type and occurrence of kernel damage in rice. *Australian Journal of Agricultural Research*, 42, 485–496.
- Terashima, K., Saito, Y., Sakai, N., Watanabe, T., Ogata, T., & Akita, S. (2001). Effects of high air temperature in summer of 1999 on ripening and grain quality of rice. *Japanese Journal of Crop Science*, 70, 449–458\*.
- Tsukaguchi, T., Nitta, S., & Matsuno, Y. (2016). Cultivar differences in the grain protein accumulation ability in rice (*Oryza sativa* L.). *Field Crops Research*, 192, 110–117.
- Tsukaguchi, T., Ohashi, K., Sakai, H., & Hasegawa, T. (2011). Varietal difference in the occurrence of milky white kernels in response to assimilate supply in rice plants (*Oryza sativa* L.). *Plant Production Science*, 14, 111–117.
- Tsukaguchi, T., Yamamura, T., Inoue, H., Nakagawa, H., Murakami, K., & Kita, E. (2012). The response of the occurrence of milky white kernels with different cross-sectional patterns of chalkiness in the endosperm to grain-filling temperature and to assimilate supply in Koshihikari. *Japanese Journal of Crop Science*, 81, 267–274\*.
- Wada, M. 1981. Studies on the effect of nitrogenous nutrition at vegetative lag phase on growth and yield of rice in southern Japan. *Bulletin of the Kyushu National Agricultural Experimental Station*, 21, 113–250\*\*.
- Wakamatsu, K., Sasaki, O., Uezono, I., & Tanaka, A. (2008). Effect of the amount of nitrogen application on occurrence of white-back kernels during ripening of rice under high-temperature conditions. *Japanese Journal of Crop Science*, 77, 424–433\*.
- Xiong, D., Yu, T., Lin, X., Fahad, S., Peng, S., Li, Y., & Huang, J. (2015). Sufficient leaf transpiration and nonstructural carbohydrates are beneficial for high-temperature tolerance in three rice (*Oryza sativa*) cultivars and two nitrogen treatments. *Functional Plant Biology*, 42, 347–356.
- Yoshida, H., Takehisa, K., Kojima, T., Ohono, H., & Nakagawa, H. (2016). Modeling the effect of N application on growth, yield and plant properties associated with the occurrence of chalky grains of rice. *Plant Production Science*, 19, 34–42.

\*In Japanese with English abstract

\*\*In Japanese