



Cultivar differences in the grain protein accumulation ability in rice (*Oryza sativa* L.)



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ABSTRACT

The demand for rice grain protein content (GPC) differs in different regions of the world. Despite large differences in GPC among cultivars, evaluation of the effects of genotype on GPC is difficult because GPC is influenced not only by cultivar traits (such as nitrogen uptake ability, sink size and heading date) but also by the environment. We hypothesized that grain protein accumulation ability (GPA) also affects GPC. The objective of this study was to clarify the differences in GPA among six lodging-tolerant, high-yielding Japanese cultivars: Bekoaoaba, Habataki, Takanari, Hokuriku193, Momiroman, and Akenohoshi. To produce a wide variation in nitrogen availability per unit sink capacity (Nav), we used nitrogen topdressing at heading and spikelet-thinning treatment. In each cultivar, we found a logarithmic relation between GPC and Nav: $GPC = A \times \ln(Nav) + B$, where A is the regression coefficient and B is a constant. A highly significant difference in regression coefficients among cultivars was found ($P < 0.01$). The regression coefficient was considered to be a measure of GPA; it varied from 0.969 in Bekoaoaba to 1.820 in Takanari. This relation suggests that GPC is determined by Nav and GPA and that the environment affects GPC through Nav. GPA is a good criterion for evaluation of the effects of genotype on GPC. Nitrogen harvest index was highly significantly explained by multiple regression with GPA and the ratio of sink capacity to total dry matter production as independent variables, suggesting the influence of GPA on plant nitrogen dynamics during the grain-filling period. Therefore, it would be useful to determine the cultivars' GPA values for optimizing nitrogen management.

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1. Introduction

Rice is the staple food for nearly half of the world's population, primarily in Asia (Mohanty, 2013), including many developing countries. Rice is an important source of proteins and calories. Although nitrogen fertilization may increase rice grain protein content (GPC), development of high-GPC cultivars is expected to ensure consistently high GPC (Hillerislambers et al., 1973; Gomez and De Datta, 1975). However, protein content affects the texture of cooked rice, increases hardness, and reduces stickiness (Hamaker and Griffin, 1990; Martin and Fitzgerald, 2002). In Japan, where sticky and tender cooked rice is favored, high GPC is considered to negatively affect rice eating quality (Matsue et al., 2001) and is not desirable. Thus, there are diverse demands in terms of rice GPC in different regions of the world.

Different cultivars differ in GPC (Perez et al., 1996; Tirol-Padre et al., 1996; Singh et al., 1998; Koutroubas and Ntanos, 2003). GPC is also affected by the nitrogen application rate, nitrogen application method, and other cultivation practices, such as planting density and weed control (De Datta et al., 1972; Gomez and De Datta, 1975). GPC in rice is correlated with plant nitrogen concentration at various growth stages (Mori et al., 2010). There are genotypic differences in the responses of GPC to nitrogen levels (Perez et al., 1996). Highly significant effects of interactions between genotype and environment or management on GPC have been reported (Perez et al., 1996; Tirol-Padre et al., 1996; Singh et al., 1998; Koutroubas and Ntanos, 2003). GPC may be affected by genotypic differences in nitrogen uptake ability at a given soil nitrogen availability, and by the ability to incorporate the absorbed nitrogen and accumulate storage protein in seeds. The grain protein accumulation ability (GPA) is a characteristic independent of plant nitrogen status and thus can be a stable criterion for the evaluation of the effects of genotype on GPC. However, to the best of our knowledge, no evaluation of the effects of genotype on GPA has been reported.

GPA is important for determination of grain quality and grain yield. The potential capacity of the sink to accumulate assimilates

Abbreviations: GPC, grain protein content; GPA, grain protein accumulation ability; Nav, nitrogen availability per unit sink capacity.

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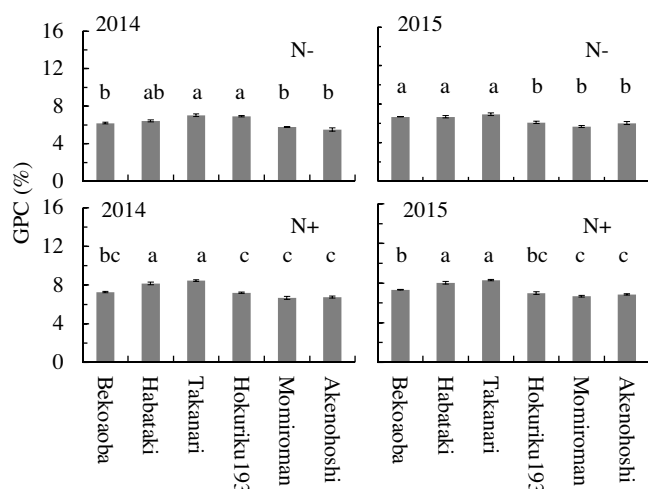


Fig. 1. Grain protein content (GPC) of intact plants with (N+) or without (N-) nitrogen topdressing at heading in 2014 and 2015. Data are means \pm standard error ($n=3$). Bars with the same letters are not significantly different at the 0.05 probability level by Tukey's test.

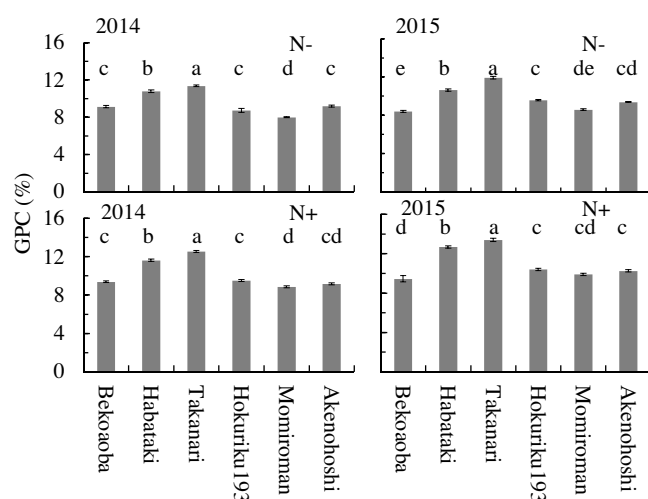


Fig. 2. Grain protein content (GPC) of spikelet-thinned plants with (N+) or without (N-) nitrogen topdressing at heading in 2014 and 2015. Data are means \pm standard error ($n=3$). Bars with the same letters are not significantly different at the 0.05 probability level by Tukey's test.

is suggested to be a measure of sink strength (Marcelis, 1996); therefore, GPA can be a good measure of sink strength for nitrogen. GPA may affect nitrogen dynamics during the grain-filling period and remobilization of nitrogen from leaves, which reduces photosynthetic ability. Therefore, characterization of genotypes with different GPA may be useful for optimum nitrogen management for each cultivar.

In this study, we hypothesized that GPC is determined by GPA and the amount of nitrogen available for developing grain per unit sink capacity (Nav) and GPA was defined as the regression coefficient between GPC and logarithm of Nav. The total amount of nitrogen available for grain is the sum of the amount of new uptake during the grain-filling period and the amount which can be remobilized from leaves (Yoshida et al., 2016). Sink capacity affects nitrogen dynamics during the grain-filling period (Wada and Wada, 1991; Ida et al., 2009). The objective of this study was to determine and compare GPA among six high-yielding, lodging-tolerant cultivars with different GPC. To produce a wide variation in Nav, nitrogen topdressing at heading and spikelet thinning were conducted.

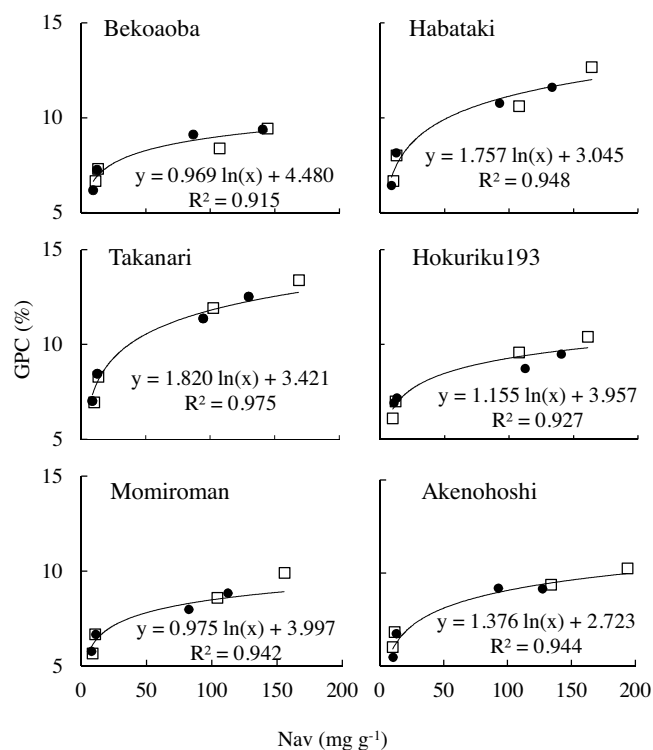


Fig. 3. The relation between nitrogen availability per unit sink capacity (Nav) and grain protein content (GPC). Solid circles, 2014; open squares, 2015.

2. Materials and methods

2.1. Plant materials

Field experiments were conducted at Ishikawa Prefectural University, Nonouchi, Japan (36°02'N, 140°04'E) in 2014 and 2015, in Gray Lowland soil. Six Japanese rice (*Oryza sativa* L.) cultivars with different genetic backgrounds – Bekoaoba, Habataki, Takanari, Hokuriku193, Momiroman, and Akenohoshi – were grown under irrigation. These are high-yielding lodging-tolerant multipurpose cultivars that have been bred by crossing *japonica* and *indica* cultivars. Bekoaoba, Momiroman, and Akenohoshi are *japonica*-dominant, whereas Habataki, Takanari, and Hokuriku193 are *indica*-dominant (Yamamoto et al., 2010).

Seeds were sown in a seedling nursery box. In 2014, 21-day-old seedlings of Bekoaoba, Habataki, and Takanari (early cultivars) and 35-day-old seedlings of Hokuriku193, Momiroman, and Akenohoshi (late cultivars) were transplanted on 23 May. In 2015, 25-day-old seedlings of early cultivars and 35-day-old seedlings of late cultivars were transplanted on 22 May. One seedling per hill was transplanted (22.2 hills m^{-2} ; 15 cm between hills; 30 cm between rows). Late cultivars were sown earlier to ensure harvesting before rainy and cold weather starts. All cultivars received a total of 8 g $N m^{-2}$ (4 g m^{-2} as basal dressing at puddling and 4 g m^{-2} as topdressing at panicle formation stage, 18–20 days before heading). At heading, half of each plot received 4 g $N m^{-2}$ as topdressing (N+) while the other half did not (N-). Nitrogen was applied as ammonium sulfate. Phosphorus (10 g m^{-2} as calcium superphosphate) and potassium (10 g m^{-2} as potassium chloride) were also applied to all plots as basal fertilizers. Weeds, insects, and diseases were controlled with standard chemicals as necessary. The experimental plots (18 m^2) were arranged in a split-plot design (main plots and subplots were cultivar and nitrogen topdressing, respectively) with three replicates.

2.2. Measurement of dry matter production and nitrogen uptake

Plants were sampled at the full-heading stage and at maturity. Maturity was regarded as the date at which more than 95% of spikelets became yellow in cultivars except Habataki and Takanari. In Habataki and Takanari, the date at which most leaves senesced was regarded as maturity because it was earlier than the date at which more than 95% of spikelets became yellow. Twelve plants were harvested from each plot. Ten plants were dried for 72 h at 80 °C and weighed. Two plants with the average number of panicles were separated into the leaf blade, leaf sheath + culm, panicle, and dead parts, which were all dried as above and weighed.

Each dried sample was ground to a powder with a cyclone sample mill (CSM-F1; Udy Co., CO, USA) with a 0.5-mm screen. The nitrogen content was measured by the Dumas combustion method (NCH Analyzer, Sumika Chemical Analysis Service, Tokyo, Japan).

2.3. Measurement of yield, yield components, and grain protein content

At maturity, plants were harvested from about 1 m² of each plot for the determination of yield and yield components. Panicles were counted and threshed, and rough grain weight was measured. The number of spikelets corresponding to 100 g of rough grain and the number of spikelets per unit area were estimated. The proportion of filled spikelets was determined by using NaCl solution of specific gravity 1.06; spikelets were considered filled if their specific gravity exceeded this value. Single-filled-grain weight was determined and adjusted to 14% moisture content. Sink capacity was determined as single-grain weight of filled spikelets multiplied by the number of spikelets per area. Nav (mg g⁻¹) was calculated by the following equation:

$$Nav = \frac{1.6(NL_H - 0.005WL_H) + \Delta N_{HtoM}}{SinkCapacity} \times 10^3$$

where NL_H and WL_H represent the leaf nitrogen content (g m⁻²) and leaf dry weight at heading stage, respectively, and ΔN_{HtoM} the nitrogen uptake during the period from heading to maturity (g m⁻²). The constant 1.6 was used to estimate the total amount of available nitrogen from leaf nitrogen content (Yoshida et al., 2016). The constant 0.005 represents the nitrogen content remaining in dead leaves.

Filled grain was then ground to a powder, and nitrogen content was measured. The protein content was calculated by multiplying the nitrogen content by 5.95 (Jones, 1941). GPC was adjusted to 14% moisture content. Harvest index (HI) was calculated as the fraction of grain dry weight relative to total above ground dry weight at maturity. Nitrogen harvest index (NHI) was calculated as the fraction of nitrogen in grain relative to the total above ground plant nitrogen content at maturity.

2.4. Spikelet-thinning treatment

At full heading (the day when 80% of all panicles headed), primary rachis branches except the uppermost and the second ones in 2014 and those except the uppermost one in 2015 were removed from all panicles of eight neighboring plants in each subplot. Plants were harvested at maturity. Dry weight, nitrogen content, and GPC were measured as in intact plants.

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA) according to the split-plot design to assess cultivar differences, the effects of nitrogen topdressing at heading, and the effects of cultivar × nitrogen interactions. For

each cultivar, the significance of the differences between mean values was analyzed using Tukey's test ($P < 0.05$). Multiple regression analysis was conducted to determine the contribution of GPA to NHI. The homogeneity of regression coefficients between GPC and Nav was tested according to Gomez and Gomez (1976).

3. Results

The heading dates were 2 Aug to 22 Aug in 2014 and 29 July to 15 Aug in 2015 (Table 1). The range of mean temperatures during the grain-filling period was 21.4–24.5 °C in 2014 and 22.0–25.7 °C in 2015. The grain-filling period was shorter in Habataki and Takanari due to early leaf senescence. The amount of solar radiation was 419–540 MJ m⁻² in 2014 and 412–511 MJ m⁻² in 2015. Mean temperature tended to be lower and the amount of radiation during the grain-filling period tended to be higher for cultivars with later heading.

Dry matter production during the whole growth period averaged over treatments was 1249–1817 g m⁻² in 2014 (Table 2) and 1142–1631 g m⁻² in 2015 (Table 3); in both years, this parameter was highest in Hokuriku193 and lowest in Bekoaoba. Nitrogen topdressing at heading increased dry matter production by 67 g m⁻² in 2014 and 81 g m⁻² in 2015. Dry matter production during the grain-filling period was 633–823 g m⁻² in 2014 and 525–705 g m⁻² in 2015. Single-grain weight was approximately 50% larger in Bekoaoba than in other cultivars in both years, whereas the number of spikelets was 40% smaller (Tables 2 and 3). Sink capacity was highest in Momiroman and lowest in Bekoaoba and Habataki in both years. Hulled grain yield was 719–832 g m⁻² in 2014 and 679–808 g m⁻² in 2015; differences among cultivars in hulled grain yield were much smaller than differences in dry matter production.

Nitrogen topdressing at heading increased nitrogen uptake during the grain-filling period by 2.9 g m⁻² in 2014 (Table 4) and 2.6 g m⁻² in 2015 (Table 5). There was no significant cultivar difference in nitrogen uptake during the grain-filling period. Nitrogen topdressing at heading increased nitrogen uptake during the whole growth period by 2.9 g m⁻² in 2014 (Table 4) and 2.8 g m⁻² in 2015 (Table 5). This parameter was highest in Hokuriku193 in 2014 and in Hokuriku193 and Takanari in 2015 and was lowest in Bekoaoba in both years. In both years, HI varied widely, 39–51% in 2014 and 42–51% in 2015. In both years, HI was highest in Bekoaoba and lowest in Hokuriku193 (Tables 4 and 5). There was no significant effect of nitrogen topdressing at heading on HI. NHI also varied widely among cultivars, 55–72% in 2014 and 62–72% in 2015. In both years, NHI was highest in Takanari and lowest in Hokuriku193 and Akenohoshi. There was no significant effect of nitrogen topdressing at heading on NHI.

There were highly significant differences in GPC among cultivars in both years (Tables 4 and 5, and Fig. 1). GPC of intact plants was in the range of 6.4–7.7% in 2014 and 6.2–7.6% in 2015. GPC was highest in Takanari and lowest in Momiroman in both years. Nitrogen topdressing at heading significantly increased GPC (by 1.2% in 2014 and 1.0% in 2015). Interaction between cultivars and nitrogen topdressing was highly significant ($P < 0.001$ in both years). The differences in GPC among cultivars were increased by nitrogen topdressing, and the difference between Takanari, the highest, and Momiroman, the lowest, was about 1.3% in both years.

Spikelet thinning markedly increased GPC and cultivar differences. GPC of spikelet-thinned plants was 8.0–12.5% in 2014 and 8.4–13.4% in 2015 (Fig. 2). GPC was highest in Takanari and lowest in Momiroman and Bekoaoba, with a difference of about 3% in both years.

In each cultivar, there was a logarithmic relation between GPC and Nav, and the coefficients of determination were higher than 0.915 and highly significant (each $P < 0.01$; Fig. 3). In the regression

Table 1
Dates of heading and maturity, duration of grain filling, and meteorological data.

	Heading	Maturity	Grain-filling duration (days)	Mean air temperature (°C)		Solar radiation (MJ m ⁻²)	
				T-H	H-M	T-H	H-M
2014							
Bekoaoba	2-Aug	18-Sep	47	23.7	24.5	932	481
Habataki	7-Aug	19-Sep	43	24.0	23.9	985	419
Takanari	13-Aug	24-Sep	42	24.0	23.4	1017	465
Hokuriku193	14-Aug	1-Oct	48	24.0	23.0	1028	526
Momiroman	14-Aug	1-Oct	48	24.0	23.0	1028	526
Akenohoshi	22-Aug	14-Oct	53	24.3	21.4	1111	540
2015							
Bekoaoba	29-Jul	7-Sep	40	23.1	25.7	796	449
Habataki	1-Aug	7-Sep	37	23.3	25.6	832	412
Takanari	5-Aug	16-Sep	42	23.5	24.4	897	421
Hokuriku193	10-Aug	24-Sep	45	23.9	23.3	975	415
Momiroman	13-Aug	7-Oct	55	24.0	22.2	1007	511
Akenohoshi	15-Aug	7-Oct	53	24.0	22.0	1024	494

T: transplanting, H: heading, M: maturity.

Table 2
Dry matter production during the whole growth period (DMP-WG) and grain-filling period (DMP-GF), yield, and yield components in 2014.

		DMP-WG (g m ⁻²)	DMP-GF (g m ⁻²)	Spikelet number × 10 ³ (m ⁻²)	Single-grain weight (mg)	Sink capacity (g m ⁻²)	Hulled grain yield (g m ⁻²)
Bekoaoba	N–	1214	601	23.11	33.0	762	718
	N+	1284	671	23.11	35.0	808	772
Habataki	N–	1365	614	39.55	19.9	786	717
	N+	1402	651	39.20	20.0	783	720
Takanari	N–	1526	672	41.21	21.7	894	811
	N+	1551	697	41.39	21.6	893	828
Hokuriku193	N–	1748	754	37.09	23.0	853	797
	N+	1885	891	38.86	22.9	891	857
Momiroman	N–	1565	701	39.91	23.8	950	812
	N+	1637	773	41.67	23.8	993	852
Akenohoshi	N–	1586	623	36.74	22.1	813	788
	N+	1649	686	38.85	22.2	863	802
Results of ANOVA							
Bekoaoba		1249d	636c	23.11b	34.0a	785b	745b
Habataki		1384c	633c	39.38a	19.9d	785b	719b
Takanari		1539b	685b	41.30a	21.6c	894a	820a
Hokuriku193		1817a	823a	37.98a	23.0b	872a	827a
Momiroman		1601b	737b	40.79a	23.8b	971a	832a
Akenohoshi		1618b	655bc	37.79a	22.2c	838ab	795b
Cultivar		**	*	***	***	***	**
	N–	1501	661	36.27	23.9	843	774
	N+	1568	728	37.18	24.3	872	805
Nitrogen		***	***	ns	ns	ns	*
Cultivar × Nitrogen		*	*	ns	ns	ns	**

Ns: not significant by ANOVA. Values with the same letter are not significantly different at the 0.05 probability level by Tukey's test.

- * Significant at the 0.05 level.
- ** Significant at the 0.01 level.
- *** Significant at the 0.001 level.

equation $GPC = A \times \ln(N_{av}) + B$, A is the regression coefficient and B is a constant. The A values varied widely among cultivars, from 0.969 in Bekoaoba to 1.820 in Takanari. A test for homogeneity of regression coefficients revealed a highly significant difference in the regression coefficients among cultivars ($P < 0.01$; Table 6).

Multiple regression analysis was conducted to determine the contribution of GPA to NHI (Table 7). With GPA and the ratio of sink capacity to dry matter production as independent variables, the overall regression was highly significant with the coefficient of determination of 0.801 in 2014 and 0.716 in 2015 ($P < 0.001$ in 2014, $P < 0.01$ in 2015, $n = 12$). Regression coefficients for both GPA and the ratio of sink capacity to dry matter production were significant. Partial correlation coefficients for GPA were 0.544 in 2014 and 0.627 in 2015, whereas those for the ratio of sink capacity to dry matter production were 0.736 in 2014 and 0.679 in 2015 (Table 7). However, with GPC as an independent variable instead of GPA, the partial correlation coefficient for GPC was not significant, although

the overall regression was significant with coefficients of determination of 0.609 in 2014 and 0.552 in 2015 ($P < 0.05$, $n = 12$, both years; Table 8).

4. Discussion

The cultivar difference in GPC varied between N application rates and years. For example, in the N– plots in 2014, GPC was higher in Hokuriku193 than in Akenohoshi but not significantly different between Hokuriku193 and Takanari, whereas GPC was lower in Hokuriku193 than in Takanari and there was no significant difference between Hokuriku193 and Akenohoshi in N+ plots in 2014 and 2015 (Fig. 1). The highly positive interactions between cultivar and nitrogen management (Tables 4 and 5) are in good agreement with previous studies (Perez et al., 1996; Singh et al., 1998; Koutroubas and Ntanos, 2003). The marked increase in GPC by spikelet-thinning treatment (Figs. 1 and 2) supports the effects of sink capacity on

Table 3
Dry matter production during the whole growth period (DMP-WG) and grain-filling period (DMP-GF), yield, and yield components in 2015.

		DMP-WG (g m ⁻²)	DMP-GF (g m ⁻²)	Spikelet number × 10 ³ (m ⁻²)	Single-grain weight (mg)	Sink capacity (g m ⁻²)	Hulled grain yield (g m ⁻²)
Bekoaoaba	N-	1095	495	21.34	32.9	701	641
	N+	1188	588	22.04	34.7	765	718
Habataki	N-	1256	495	35.95	20.2	726	682
	N+	1317	556	36.60	20.1	736	695
Takanari	N-	1349	538	38.25	22.2	848	773
	N+	1423	612	39.20	21.5	844	804
Hokuriku193	N-	1556	611	35.02	23.9	836	750
	N+	1706	761	37.98	23.7	901	833
Momiroman	N-	1515	672	36.64	26.3	964	791
	N+	1581	738	38.12	26.1	994	824
Akenohoshi	N-	1430	527	36.45	22.1	805	712
	N+	1470	566	37.91	21.7	824	718
Results of ANOVA							
Bekoaoaba		1142d	542b	21.69b	33.8a	733b	679b
Habataki		1287c	525b	36.27a	20.1e	731b	689b
Takanari		1386b	575b	38.73a	21.8d	846a	789a
Hokuriku193		1631a	686a	36.50a	23.8c	868a	791a
Momiroman		1548ab	705a	37.38a	26.2b	979a	808a
Akenohoshi		1450ab	546b	37.18a	21.9d	815ab	715b
Cultivar		**	*	***	***	***	**
	N-	1367	556	33.94	24.6	813	725
	N+	1448	637	35.31	24.6	844	765
Nitrogen		***	***	ns	ns	ns	*
Cultivar × Nitrogen		*	*	ns	ns	ns	**

Ns: not significant by ANOVA. Values with the same letter are not significantly different at the 0.05 probability level by Tukey's test.

* Significant at the 0.05 level.

** Significant at the 0.01 level.

*** Significant at the 0.001 level.

Table 4
Nitrogen uptake during the whole growth period (Nup-WG) and grain-filling period (Nup-GF), grain protein content (GPC), harvest index (HI), and nitrogen harvest index (NHI) in 2014.

		Nup-WG (g m ⁻²)	Nup-GF (g m ⁻²)	GPC (%)	HI (%)	NHI (%)
Bekoaoaba	N-	9.5	1.1	6.2	50.9	67.4
	N+	12.3	4.0	7.6	51.7	68.9
Habataki	N-	10.2	1.1	6.4	45.2	65.3
	N+	13.3	4.2	8.2	44.2	63.9
Takanari	N-	11.3	1.1	7.0	45.7	72.7
	N+	14.3	4.1	8.4	45.9	70.6
Hokuriku193	N-	14.3	1.4	6.9	39.2	55.6
	N+	16.8	3.9	7.4	39.1	54.2
Momiroman	N-	11.2	1.3	5.8	44.6	60.7
	N+	14.3	4.5	7.0	44.8	59.9
Akenohoshi	N-	11.6	1.3	6.1	42.7	59.8
	N+	14.3	4.0	7.2	41.8	58.0
Results of ANOVA						
Bekoaoaba		10.9c	2.6	6.9b	51.3a	68.2b
Habataki		11.7bc	2.6	7.3a	44.7b	64.6bc
Takanari		12.8b	2.6	7.7a	45.8b	71.6a
Hokuriku193		15.6a	2.7	7.1ab	39.2c	54.9d
Momiroman		12.7b	2.9	6.4c	44.7b	60.3c
Akenohoshi		12.9b	2.7	6.6b	42.3bc	58.9cd
Cultivar		**	ns	***	***	***
	N-	11.3	1.2	6.4	44.7	63.6
	N+	14.2	4.1	7.6	44.6	62.6
Nitrogen		***	***	***	ns	ns
Cultivar × Nitrogen		ns	ns	***	ns	ns

Ns: not significant by ANOVA. Values with the same letter are not significantly different at the 0.05 probability level by Tukey's test.

** Significant at the 0.01 level.

*** Significant at the 0.001 level.

GPC, suggesting an association of nitrogen availability per unit sink mass with GPC. The negative correlation between GPC and grain yield (Gomez and De Datta, 1975; Simmonds, 1995) may reflect the positive effect of sink capacity on grain yield and its negative effect on Nav.

We found a logarithmic relation between GPC and Nav, with different regression coefficients (GPA) for different cultivars (Fig. 3).

The environment may affect GPC through Nav, and differing relationships between environment such as soil nitrogen level and Nav for each genotype may explain the interaction between genotype and environment for GPC. Nitrogen uptake ability is one of the major traits determining Nav and is affected by root architecture, morphology, transporter activity and carbon availability (Xu et al., 2012). There is wide genotypic variation in nitrogen uptake ability

Table 5

Nitrogen uptake during the whole growth period (Nup-WG) and grain-filling period (Nup-GF), grain protein content (GPC), harvest index (HI), and nitrogen harvest index (NHI) in 2015.

		Nup-WG (g m ⁻²)	Nup-GF (g m ⁻²)	GPC (%)	HI (%)	NHI (%)
Bekoaoba	N-	10.6	2.0	6.7	50.4	67.7
	N+	12.9	4.4	7.3	51.9	68.6
Habataki	N-	11.3	2.2	6.7	46.7	67.5
	N+	14.5	4.7	8.0	45.4	64.8
Takanari	N-	12.2	2.3	6.9	49.3	73.8
	N+	15.8	4.9	8.3	48.6	71.1
Hokuriku193	N-	12.3	1.5	6.1	41.4	62.4
	N+	15.4	4.4	7.0	42.0	63.7
Momiroman	N-	11.7	1.8	5.7	44.9	64.2
	N+	14.1	4.5	6.7	44.9	65.8
Akenohoshi	N-	11.4	1.7	6.0	42.8	63.3
	N+	13.7	4.0	6.8	42.0	60.0
Results of ANOVA						
Bekoaoba		11.7b	3.2	7.0b	51.1a	68.1b
Habataki		12.9ab	3.5	7.3a	46.1b	66.1b
Takanari		14.0a	3.6	7.6a	49.0ab	72.4a
Hokuriku193		13.8a	3.0	6.5b	41.7c	63.0c
Momiroman		12.9ab	3.1	6.2c	44.9bc	65.0bc
Akenohoshi		12.6ab	2.9	6.4b	42.4c	61.7c
Cultivar		**	ns	***	***	**
Nitrogen	N-	11.6	1.9	6.3	45.9	66.5
	N+	14.4	4.5	7.3	45.8	65.7
Cultivar × Nitrogen		ns	ns	***	ns	ns

Ns: not significant by ANOVA. Values with the same letter are not significantly different at the 0.05 probability level by Tukey's test.

** Significant at the 0.01 level.

*** Significant at the 0.001 level.

Table 6

The regression coefficient between GPC and logarithm of Nav of the six cultivars.

	GPA
Bekoaoba	0.969
Habataki	1.757
Takanari	1.820
Hokuriku193	1.155
Momiroman	0.975
Akenohoshi	1.376
F-value	8.66**

in rice (Perez et al., 1996; Singh et al., 1998; Koutroubas and Ntanos, 2003; Yoshinaga et al., 2013) and the response of nitrogen uptake to different soil nitrogen availability may differ among genotypes (cultivars). Sink capacity is genetically determined but is also highly

influenced by the environment. There is also wide genotypic variation in sink production efficiency, i.e. sink capacity per nitrogen uptake at full heading (Yoshida et al., 2006; Yoshinaga et al., 2013), which directly affects Nav. Nav may be affected by the environment through grain weight. High radiation intensity during grain-filling period increases single-grain weight (Yoshida and Hara, 1977), which would decrease Nav. There is a cultivar difference in the radiation use efficiency in rice (Takai et al., 2006) and there may be a cultivar difference in the relation between assimilate availability and grain weight. The environment may affect Nav through differences in the response of growth duration to daylength or temperature. Late-maturing cultivars intercept larger amounts of solar radiation and produce more dry matter, which reduces plant nitrogen concentration, resulting in lower GPC than in early-maturing cultivars (Hillerislambers et al., 1973; Perez et al., 1996). The wide

Table 7

Multiple regression analysis with NHI as a dependent variable and GPA and the ratio of sink capacity to dry matter production in the whole growth period (S/D ratio) as independent variables.

	F-value	R ²	Regression coefficient			Partial correlation coefficient	
			Intercept	GPA	S/D ratio	GPA	S/D ratio
2014	18.6***	0.801	4.56	8.675**	0.826**	0.544	0.736
2015	11.35**	0.716	20.63	6.460**	0.618**	0.627	0.679

** Significant at the 0.01 level.

*** Significant at the 0.001 level.

Table 8

Multiple regression analysis with NHI as a dependent variable and GPC and the ratio of sink capacity to dry matter production in the whole growth period (S/D ratio) as independent variables.

	F-value	R ²	Regression coefficient			Partial correlation coefficient	
			Intercept	GPC	S/D ratio	GPC	S/D ratio
2014	7.02*	0.609	-0.574	14.09	0.835**	0.323	0.744
2015	5.55*	0.552	17.488	14.06	0.548*	0.468	0.603

* Significant at the 0.05 level.

** Significant at the 0.01 level.

variation in GPC due to different environments and management practices can be well accounted for by the variation in Nav.

Although there was only a small cultivar difference in GPC at low Nav, the difference became larger as Nav increased; consequently, there was a wide difference ($P < 0.01$) in the regression coefficient A among cultivars (Fig. 3 and Table 6). The regression coefficient A indicates the increment in GPC in response to the increment in the logarithm of Nav: higher A values show higher GPC at a given Nav. Therefore, the regression coefficient A represents the intrinsic ability of grain to accumulate protein, or GPA.

GPC may be affected by temperature during grain-filling period directly or indirectly. High temperature shortens grain-filling period and reduces the amount of assimilate available for developing grain and thus grain weight (Yoshida and Hara, 1977), which would increase Nav because a large part of total amount of nitrogen available for developing grain would be determined by the grain-filling period. As a result, GPC would be increased but the regression coefficient in the relation between Nav and GPC would not be affected by temperature unless temperature directly affects grain protein accumulation. Yamakawa et al. (2007) revealed that high temperature during grain-filling period reduced accumulation of some storage protein but the high temperature used was far beyond the optimum temperature (32/28 °C, day/night). Thus the direct effect of temperature on the regression coefficient is unknown in the optimum or suboptimum temperature range.

GPA affected plant nitrogen dynamics during the grain-filling period. NHI was explained well by multiple regression with GPA and the ratio of sink capacity to dry matter production as independent variables (Table 7). GPA can be considered as the sink strength for nitrogen and the ratio of sink capacity to dry matter production as the relative sink size. The high NHI indicates a high proportion of nitrogen accumulated in grain relative to that remaining in the vegetative parts at maturity. Although NHI represents only the ultimate result of plant nitrogen dynamics, it indicates the involvement of GPA in plant nitrogen dynamics. The higher GPA means higher allocation of nitrogen to grain, i.e. a higher proportion of nitrogen acquired during the grain-filling period distributed to developing grain or a larger amount of nitrogen remobilized from vegetative organs to grain. However, the partial correlation coefficient for the effect of GPC on NHI was not significant (Table 8). GPC is only a resultant of Nav and GPA and does not represent sink strength for nitrogen.

The importance of GPA in nitrogen dynamics increases in high-yielding cultivars, whose nitrogen uptake is generally higher than that of standard cultivars (Ookawa et al., 2003; Yoshinaga et al., 2013). Sink production efficiency is larger in high-yielding cultivars (Yoshida et al., 2006; Yoshinaga et al., 2013). High-yielding cultivars with large sink capacities require more nitrogen during the grain-filling period than standard cultivars. This higher demand for nitrogen is met by remobilizing nitrogen from vegetative parts (Sheehy et al., 2004). The amount of nitrogen remobilized from leaves to panicles is larger in rice cultivars with larger sink size (Wada and Wada, 1991; Ida et al., 2009). Nitrogen remobilization from leaves decreases photosynthetic capacity, because approximately 80% of total leaf nitrogen in rice plants is invested in chloroplasts (Mae and Ohira, 1982), and the synthesis and amount of Rubisco reflect plant nitrogen status (Imai et al., 2008). Therefore, adequate nitrogen management according to GPA of each cultivar is necessary to make the best use of its yield potential. This is especially important for high-yielding cultivars because a small increase in GPA would result in a greater increase in total nitrogen demand in cultivars whose sink capacity is greater than in standard cultivars.

GPA can be a good criterion for evaluating genotypes for GPC because GPA is genotype-specific and is unaffected by soil nitrogen availability. GPC as such is not suitable for genotype evaluation

because its heritability is low (Hillerislambers et al., 1973) possibly due to its dependence on Nav. Although some QTLs for GPC have been reported (Tan et al., 2001; Hu et al., 2004), such QTLs should also include QTLs for traits that affect sink capacity and nitrogen uptake, such as spikelet number, grain size, and root profile, because these traits are indirectly associated with GPC. Some of such QTLs may not be detected under certain environments or nitrogen managements. Ye et al. (2010) compared GPC of 21 single-chromosome substitution lines in 8 environments and found a highly significant interaction between substitution and environment. Some substitutions had a large positive effect in one environment but no or negative effect in another. It would be very interesting to determine which of the substitutions is associated with GPA.

High GPA can be targeted in breeding programs for regions where high GPC is preferable from the point of view of nutrition. However, high GPA does not guarantee high GPC. Even in high-GPA cultivars, sufficient Nav is necessary to attain high GPC. Maintaining Nav at a certain level requires sufficient nitrogen uptake during the grain-filling period and in some cases also the control of sink capacity at the expense of yield. Nevertheless, high GPA results in efficient grain accumulation of protein synthesized using absorbed nitrogen. In regions where low GPC is preferred from the point of view of eating quality, such as Japan, low GPA can be targeted. Appropriate nitrogen management for cultivars with low nitrogen demand for grain may enable high yields with relatively low nitrogen input, which would reduce the environmental burden and cultivation cost.

In conclusion, we found that GPC is determined by Nav (nitrogen availability per unit sink capacity) and GPA (the regression coefficient between GPC and logarithm of Nav). GPA is a cultivar-specific parameter and a measure of sink strength for nitrogen, because it affects plant nitrogen dynamics during the grain-filling period. Because GPA does not depend on plant nitrogen status or sink capacity, it would be a good trait for evaluation of the effects of genotype on GPC. Furthermore, GPA is an important trait for optimization of the nitrogen management method for each cultivar.

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