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Controlling Yield and Grain Protein Content of Wheat in Japan through Pre-Anthesis Nitrogen Application to Maximize Producers' Profit

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Abstract

Acquiring a high grain yield while maintaining the required grain protein content (GPC) level is vital for profitable wheat farming, but doing so is typically difficult as GPC is affected by climatic and soil-related factors and management. This study was conducted to investigate the applicability of N application at flowering time, with an emphasis on late sowing conditions, for adjusting GPC to expected levels without compromising the yield of hard and soft wheat in volcanic ash soils with high nitrogen supplying capacity. Two groups of field experiments were conducted between November 2010 and June 2013, with two hard wheat and two soft wheat cultivars under early, standard, and late sowing conditions with two split N regimes, wherein N was applied: as basal, at the stem elongation stage, and at flowering. Our results suggested that the N management strategy at flowering time for increasing GPC is suitable under optimum sowing conditions, and that the GPC of both hard and soft wheat sown at an optimum timing can be adjusted for fitting into the quality bonus window, by altering the fertilizer application rate at flowering time. This study contributes toward improving our understanding of the effects of split N fertilizer application at the stem elongation and flowering stages, and the effect of sowing time on the grain yield and GPC of both hard and soft wheat grown in volcanic ash soils.

Discipline: Crop production
Additional key words: late-sowing, quality bonus, volcanic ash soils

Introduction

Wheat is the second highest energy source after rice in the diet of Japanese (FAOSTAT, 2014). People consume wheat products such as bread, pasta, Japanese noodles (*udon*) and Chinese noodles (*ramen*) almost on a daily basis. However, the self-sufficiency ratio of wheat in Japan is a mere 13.3% (FAOSTAT 2014), and wheat is ranked second after maize among import commodities in the food and agriculture category (FAOSTAT 2014). Although Japan has favourable wheat-growing conditions, with sufficient annual precipitation of 840-2800 mm, the national average yield in Japan (4.1 t ha^{-1}) is lower than that in other major wheat-producing countries in Europe, such as France, Germany, and the UK (7.4, 8.6, and 8.6 t ha^{-1}, respectively).

To increase the self-sufficiency of wheat in Japan, the Japanese government is offering a subsidy for wheat production in the form of a "quality bonus" based on its grain-quality indices. In this system, the producers receive a special payment from the government based on four grain-quality indices: grain protein content (GPC), falling number, ash content, and bulk density.

Among these quality indices, GPC is the most variable, as it depends on soil and climatic conditions, as well as management practices. It has been widely reported that wheat GPC is influenced by the climate, cultivar, nitrogen (N) application rate, N application timing, seeding rate, soil fertility, and the interactions among these factors (Lopez-Bellido et al. 2005, Garrido-Lestache et al. 2004, Gauer et al. 1992, Sato et al. 1992, Rao et al. 1993, Nakano & Morita 2009, Nakano et al.

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2008). In addition, there is usually a negative genetic correlation between the grain yield and GPC (Triboi et al. 2006, Asseng & Milroy 2006, Kibite & Evans 1984, Loffler 1985, Selles & Zentner 2001, Fowler 2003, Triboi & Triboi-Blondel 2002), which makes it challenging to increase the yield while maintaining GPC within the target range (Triboi et al. 2006).

Wheat varieties in Japan are classified into two groups—hard wheat (used for bread) and soft wheat (used for noodles), depending on the grain quality (mainly protein content) (Nakano et al. 2008). The required grain N concentrations for hard wheat and soft wheat are 2.3-2.8% and 1.9-2.3%, respectively. As for GPC, the range is 11.5-14% for hard wheat and 9.7-11.3% for soft wheat (MAFF 2014). Wheat producers in Japan can obtain the highest quality bonus when GPC falls into this range. In other countries such as the U.S., the premium prices for wheat are also determined based on GPC (Olmos et al. 2003).

Recent research has attested that GPC can be controlled by N application at the flowering stage, while N application at the stem-elongation stage affects the grain yield (Nakano & Morita 2009, Woolfolk et al. 2002, Fischer et al. 1993, Yoshida et al. 2008, Shimazaki & Watanabe 2010, Takayama et al. 2006, Stark & Tindall 1992, Zebarth & Sheard 1992, Peltonen 1993, Bly & Woodard 2003, Rutkowska 2009, Knowles et al. 1994, Rawluk et al. 2000). However, the effectiveness of split N application at flowering for improving GPC depends on the soil type, and therefore, the control of GPC solely by split N application is difficult (Karathanasis et al. 1980, Sato et al. 1992, Nakatsuji 2003).

Volcanic ash soils, among the major soil types in Japan (Shoji & Takahashi 2002, Nanzyo 2002), are important soils for wheat production in Japan. Hokkaido, the largest wheat-growing prefecture in Japan, has a high percentage (40%) of volcanic ash soils (Shoji & Takahashi 2002), while the Kanto region, the third-largest wheat growing area, is composed of almost entirely of volcanic ash soils. Both regions collectively account for 73% of Japan's total wheat production (MAFF 2015).

Volcanic ash soils are among the most productive soils in the world for agriculture and forestry, and usually have their colloidal fraction dominated by Al-humus complex or allophane/imogolite in humid weathering environments (Ugolini & Dahlgren 2002). Organic matter tends to be highly accumulated in volcanic ash soils, owing to the stabilization afforded by the formation of Al-humus complex in such soils. Non-crystalline materials and humus contribute to the unique chemical and physical soil properties of volcanic ash soils, such as high water-holding capacity, variable charge, high phosphorus retention, low bulk density, high friability, and high rate of formation of stable soil aggregates (Shoji et al. 1993). Some of these properties support higher crop productivity through a better retention of nutrients and water, as compared with other soil types. The native nitrogen-supplying capacity in volcanic ash soils is higher than that in other soils, and is further enhanced by the application of N fertilizers that stimulates mineralization of the native soil nitrogen (Eneji et al. 2002).

In areas with a rice-wheat cropping system, wheat sowing is primarily delayed due to a late harvest coupled with poor soil structure and loose plant residues, which adversely affects preparation of the land for wheat cropping (Byerlee et al. 1984). Moreover, heavy rainfall during the land-preparation period might cause a further delay in sowing (Aslam et al. 1993, as cited in Farroq et al. 2008). The delayed sowing tends to increase GPC, while typically reducing the yield (Singh & Jain 2000). Late planting is a major limitation to wheat productivity in many regions of South Asia that have a rice-wheat cropping system (Hobbs et al. 1994). The interaction between the sowing time and the effect of fertilization at flowering time on GPC is an area that has not been duly addressed in literature, especially in relation to the wheat grown in volcanic ash soils.

The purpose of this study was to test the applicability of N application at flowering time in volcanic ash soils for controlling GPC, under the context of late sowing conditions, and to examine the possibility of introducing other varieties to stabilize the yield and GPC, even in late sowing situations.

Materials and methods

1. Experimental site

Field experiments were conducted at the Institute for Sustainable Agro-Ecosystem Services (ISAS) (35°44′N, 139°32′E), University of Tokyo, in Nishitokyo City, Tokyo. The experimental site is located on the Kanto Plain, where volcanic ash soil, classified as Typic Melanudand by USDA soil taxonomy and as Andosol by FAO soil classification, is dominant. The soil layer from the surface to a depth of 30 cm was Kuroboku andisol (Clay loam, with bulk density of 0.73-0.78 g cm^{-3}), and from a depth of 30 cm to 100 cm was Tachikawa loamy andisol (Loam, with bulk density of 0.41-0.77 g cm^{-3}) (Table 1). The initial soil NO_3^- and NH_4^+ content of the 0-15 cm top soil layer before fertilization in October 2010 were 11.9 and 19.8 mg N kg^{-1}, respectively. However, both decreased to 3.3 and 8.3 mg N kg^{-1}, respectively, in November 2012 (data not shown). The NO_3^- and NH_4^+ in the soil was extracted with H_2O and 2N KCl, respectively, and their concentrations were measured by using ion selective electrodes (Thermo Fisher Scientific, Waltham, USA). The average annual precipitation was 1530 mm and the average annual temperature was 16.5°C (with minimum and maximum monthly averages of 6.2°C and 27.8°C, respectively).

2. Plant materials

We used four wheat varieties—Yumeshiho, Ayahikari, Nishinokaori, and Nebarigoshi. Yumeshiho and Ayahikari are the recommended varieties for the Kanto area, Nishinokaori for the Western region, and Nebarigoshi for the Northern region of the main island. Yumeshiho is a hard wheat variety with a high flour yield and good bread-making quality. It has a shorter culm length and is moderately resistant to leaf rust and wheat yellow mosaic virus (Kiribuchi-Otobe et al. 2009). Ayahikari is an early maturing, high-yield soft wheat variety, which has a low amylose content that helps produce smooth noodles. It is also resistant to leaf rust and wheat yellow mosaic virus (Yoshida et al. 2001). Nishinokaori is an early maturing hard wheat variety, which has a higher GPC than the other varieties in the region, but also a slightly lower yield. It has a shorter culm length and is more resistant to lodging than the other varieties in this region (Kawase, K. 2009, Taya et al. 2003). Nebarigoshi is a soft wheat variety, but is also considered a dual-purpose variety due to its good bread-making quality (Taya et al. 2003).

3. Experiment 1: Effect of N application at flowering time (2010-2011 and 2011-2012)

(1) Experimental design and treatments

The experiment comprised two cropping seasons: 2010-2011 and 2011-2012. The experimental design was a split-plot in 2010-2011 with two sowing dates (Nov. 2 and Dec. 1) as the main factor, two wheat varieties (Ayahikari and Yumeshiho) as a sub factor, and four levels of N application (0, 80, 110, and 140 kg N ha^{-1}) as a sub-sub factor (Table 2). The 2011-2012 experiment used a split-plot design with the same two varieties as the main factor, and the same N treatments as a sub-factor, with a single sowing date (Nov. 16). In both seasons, the experiment

Table 1. Soil characteristics

Soil layer	Texture	Bulk density (g cm^{-3})	C/N*	Volumetric water content (%) at		
				Saturation	Field capacity	Permanent wilting point
Top soil - Kuroboku						
0-15 cm	Clay loam	0.73	13.7	48.2	41.9	31.6
15-30 cm		0.78	13.1	45.7	42.0	31.8
Sub soil - Tachikawa loam	Loam					
30-45 cm		0.77	13.8	46.4	44.7	33.4
45-60 cm		0.45	13.8	62.4	57.7	48.7
60-80 cm		0.41	14.5	64.5	60.2	52.4
80-100 cm		0.45	14.5	62.1	58.3	51.6

* (Kato T, 2003)

Table 2. Rates of nitrogen fertilizer application (kg N ha^{-1})

	Year	Treatment	Total N	Basal	First split (at stem elongation)	Second split (at flowering)
Experiment 1	2010-2011	N0	0	0	0	0
	and	N1	80	60	20	0
	2011-2012	N2	110	60	20	30
		N3	140	60	20	60
Experiment 2	2012-2013	N0	0	0	0	0
		N1	80	40	20	20
		N2	150	80	40	30

was conducted with three replications.

(2) Crop management

N application was split into three stages: as basal, at the stem elongation stage, and at flowering (Table 2). Ammonium sulphate was used as the N fertilizer. The plot area was 24 m^2 and 48 m^2 in 2010-2011 and 2011-2012, respectively. In both years, the plots were supplied with a PK fertilizer in the form of basal application at a sufficient level (P_2O_4 100 kg ha^{-1} and K_2O 75 kg ha^{-1}). Sowing was conducted using a non-till drill seeder, with 19-cm row spacing. The sowing density and depth were 80 kg ha^{-1} and 25 mm, respectively. The standard sowing window of the region is early to mid-November.

(3) Measurements

At physiological maturity, 1 m × 0.95 m area of each plot was sampled for the analysis of grain yield, total biomass, and harvest index. The dry matter and seed dry weight were determined by drying the samples at 80°C until a constant weight was achieved. The grain N content was analysed by the dry combustion method (Elemental Analyzer Flash EA 1112, Thermo Electron Corporation, Delft), and a conversion factor of 5.7 was used to derive GPC from the N content. The grain yield and GPC results were corrected by adjusting the moisture content to be 12.5% and 13.5%, respectively.

4. Experiment 2: Interaction effects of varieties, sowing dates, and fertilizer application rates (2012-2013)

(1) Experimental design and treatments

Experiment 2 comprised one field experiment conducted in the 2012-2013 cropping season. The experimental design was a split-split plot design, with four wheat varieties (Ayahikari, Yumeshiho, Nishinokaori, and Nebarigoshi) as the main factor, three N levels (0, 80, and 150 kg N ha^{-1} in total) as a sub factor, and four sowing dates (Oct. 17, Nov. 8, Nov. 29, and Dec. 19) as a sub-sub factor.

(2) Crop management

N application was split into three stages: as basal, at the stem elongation stage, and at flowering. Ammonium sulphate was used as the N fertilizer (Table 2). The plot area was 36 m^2. The variety Nebarigoshi was not sown on Oct. 17 due to the seeds being unavailable. Other crop management practices were the same as that in Experiment 1 (see 3 (2)).

(3) Measurements

At flowering, 0.5 m × 0.57 m area of each plot was sampled for measuring the total biomass and leaf area index (LAI). The phenology was observed every week throughout the experiment. Measurements and analysis conducted at physiological maturity were the same as in Experiment 1. Yield components were also analysed.

5. Statistical Analysis

Experimental data for each year were subjected to analysis of variance (ANOVA) and the means were compared with Tukey's test at $P < 0.05$, using R statistical analysis software (R Development Core Team 2008).

Results

1. Weather conditions

Figure 1 shows the weather conditions for the three study seasons at the experimental site, and the average of the past 30 years (1981-2010) at the nearest weather station (Tokyo District Meteorological Observatory, 22 km away). Considering the total precipitation during the wheat-growing season, 2011-2012 was the wettest (1225 mm) followed by 2010-2011 (1138 mm) and 2012-2013 (899 mm). The average over the last 30 years was 1157 mm. The precipitation distribution pattern was relatively consistent among the three seasons. However, the precipitation during March and April was low in 2010-2011 and the precipitation in early spring (March) when the maximum temperature starts increasing rapidly was

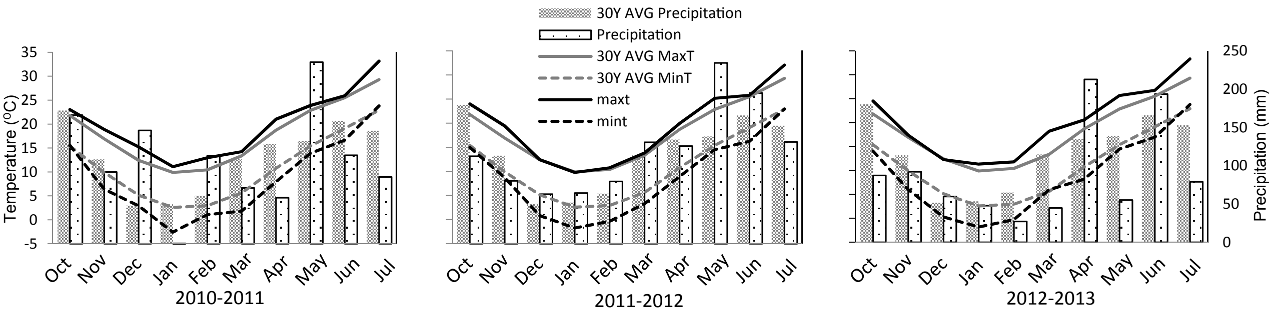


Fig. 1. Monthly average of daily maximum and minimum temperatures and monthly precipitation for three cropping seasons of the experiments, and their 30-year average at the Tokyo Meteorological Station, Tokyo, Japan

higher in 2012 compared to other years. The variation in temperature during the three growing seasons was also similar. The minimum temperature dropped to nearly zero in December in these three years.

2. Experiment 1: Effect of N application at flowering time (2010-2011 and 2011-2012)

N application at both the sowing and stem elongation stages tended to have increased the grain yield and total dry weight at physiological maturity (*cf.* N0 and N1) of both hard and soft wheat varieties during the two seasons, although the effect was clearer for total dry weight in hard wheat (Table 3). However, N application at flowering did not have a significant effect on the yield and total dry weight at maturity (*cf.* N1, N2 and N3), except for one case (showing a total dry weight increase in the Nov. 2 sowing group for Ayahikari in 2010-2011 (*cf.* N1 and N2)). Overall, the effect of N application was significant when all N treatments including N0 were compared ($P < 0.001$), but was not significant for the grain yield and total dry weight at maturity when only comparing N1-N3. The effect of sowing time × variety interaction was significant for the total dry weight ($P < 0.05$). When the analysis of variance was conducted excluding N0, there were significant effects of sowing time and sowing time × variety interaction ($P < 0.05$) in yield. There were no remarkable differences in the harvest index across the sowing times and varieties.

On the other hand, GPC increased significantly with increasing N application at flowering (*cf.* N1, N2 and N3), but not with N application at both the sowing and stem elongation stages (*cf.* N0 and N1) (Table 3). The only exception was the Yumeshiho sown on Dec. 1 in 2010 where GPC was increased only by the early N application. Sowing time × fertilizer interaction ($P < 0.001$) and variety × fertilizer interaction ($P < 0.05$) were significant with respect to GPC. When the analysis of variance was conducted excluding the zero N treatment (N0), there was a significant effect of variety as well for GPC ($P < 0.05$), but the variety × fertilizer interaction was not significant. Figure 2 shows that 30 kg of N application at flowering time (N2) increased the GPC of Yumeshiho, pushing it into the supreme quality bonus window, at both the Nov. 2. and Nov. 16 sowings, while the GPC of Ayahikari almost entered in this window under both N1 and N2 at the Nov. 2 and Nov. 16 sowings. The supply of 60 kg N

Table 3. Effect of N application rate (split) and sowing time on grain yield, GPC, total dry weight at maturity, and harvest index in 2010-2011 and 2011-2012

Year	Sowing time	Fertilizer treatment		Yield ($g\ m^{-2}$) (12.5% moisture)				GPC % (13.5% moisture)				Total dry weight ($g\ m^{-2}$)				Harvest Index	
				Ayahikari		Yumeshiho		Ayahikari		Yumeshiho		Ayahikari		Yumeshiho		Ayahikari	Yumeshiho
2010-2011	2-Nov	*N0*	0-0-0	373	b	239	b	8.9	b	10.2	b	667	b	490	b	0.49	0.42
		N1	60-20-0	501	a	498	ab	9.5	b	11.4	b	884	b	848	a	0.50	0.51
		N2	60-20-30	646	a	475	ab	11.9	a	12.8	b	1082	a	933	a	0.52	0.44
		N3	60-20-60	634	a	619	a	12.4	a	14.2	a	1091	a	1077	a	0.51	0.51
	1-Dec	*N0*	0-0-0	194	a	250	b	12.1	b	11.5	b	318	a	437	b	0.53	0.50
		N1	60-20-0	350	a	533	a	13.4	b	13.3	a	329	a	856	a	0.50	0.54
		N2	60-20-30	417	a	474	a	13.7	b	14.7	a	677	a	798	a	0.54	0.52
		N3	60-20-60	438	a	496	a	14.2	a	15.2	a	758	a	776	a	0.49	0.56
2011-2012	16-Nov	*N0*	0-0-0	229	b	193	b	11.7	b	9.4	c	340	b	317	b	0.59	0.54
		N1	60-20-0	447	a	408	a	10.7	b	10.0	c	677	a	626	a	0.58	0.57
		N2	60-20-30	425	a	424	a	11.2	b	12.8	bc	636	a	669	a	0.59	0.55
		N3	60-20-60	489	a	445	a	15.6	a	17.1	a	698	a	705	a	0.61	0.55
	ANOVA																
	Sowing (A)			NS	(*)			NS	(NS)			**	(NS)			NS	(*)
	Variety (B)			NS	(NS)			NS	(*)			*	(NS)			NS	(NS)
	Fertilizer (C)			***	(NS)			***	(***)			***	(NS)			NS	(NS)
	A x B			NS	(*)			NS	(NS)			*	(*)			NS	(NS)
	A x C			NS	(NS)			***	(***)			NS	(NS)			NS	(NS)
	B x C			NS	(NS)			*	(NS)			NS	(NS)			*	(NS)
	A x B x C			NS	(NS)			NS	(NS)			NS	(NS)			NS	(NS)

Fertilizer treatment denoted by same letters do not differ significantly (P <0.05, Tukey's test)
***,**,* : significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$ (within brackets are results excluding 0-0-0 N treatment)

at flowering time (N3) tended to increase GPC beyond the quality bonus window for both varieties, although the increase was lower for the Nov. 2 sowing than for the Nov. 16 sowing. The late sowing group (Dec. 1) tended to have a higher GPC compared with other sowing groups under zero N supply (N0) for both varieties, and the N application at flowering time tended to result in an even higher GPC, above the quality bonus range, in both varieties. Thus, the GPC of Ayahikari and Yumeshiho sown at the optimum timing is amenable for reaching the quality bonus window through fertilizer application. Therefore, the application of N at flowering is shown to be more effective more under optimum sowing conditions than under late sowing conditions.

3. Experiment 2: Interaction of variety, sowing date, and fertilizer application (2012-2013)

(1) Phenology

Figure 3 shows that both flowering and maturity were delayed by late sowing. In fact, the duration from sowing to maturity was shorter by 15-35 days with late sowing than with standard sowing (Nov. 8). The

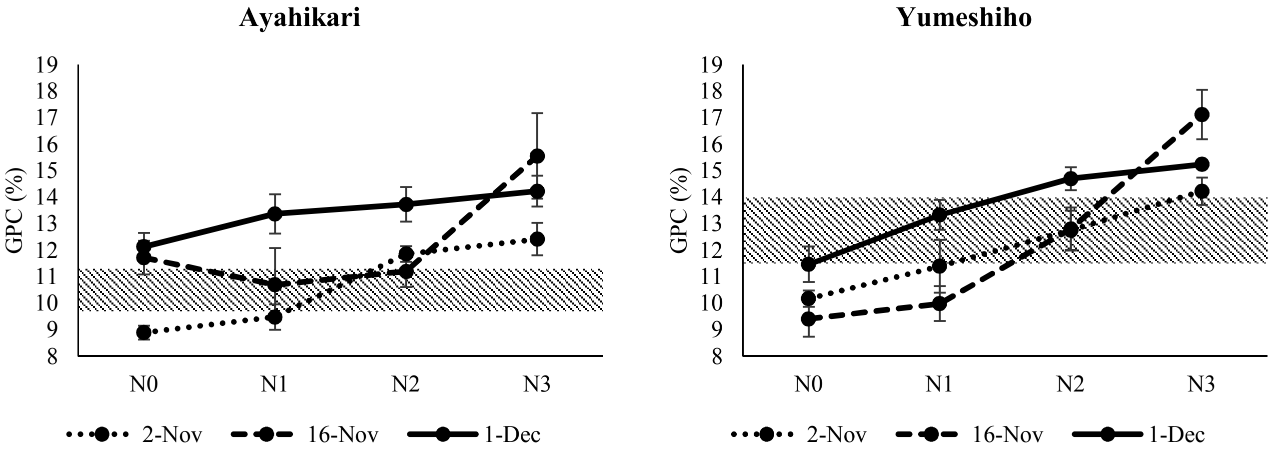
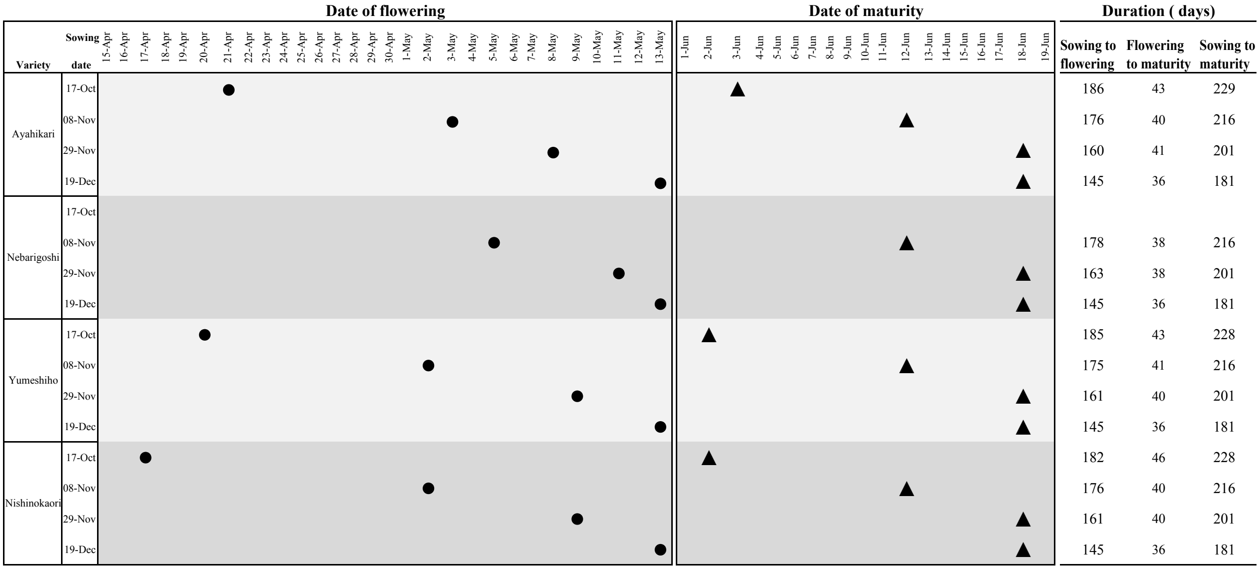


Fig. 2. GPC response of the soft wheat variety (Ayahikari) and hard wheat variety (Yumeshiho) to different N treatments at different sowing times (2010-2011 and 2011-2012 experiments) [Different lines represent different sowing dates. N treatments are N0, N1, N2, and N3 (0-0-0, 60-20-0, 60-20-30, and 60-20-60, respectively). Shaded areas show the quality bonus window for GPC (hard wheat: 11.5-14%; soft wheat: 9.7-11.3%).]



Variety	Sowing date	Sowing to flowering	Flowering to maturity	Sowing to maturity
Ayahikari	17-Oct	186	43	229
	08-Nov	176	40	216
	29-Nov	160	41	201
	19-Dec	145	36	181
Nebarigoshi	17-Oct			
	08-Nov	178	38	216
	29-Nov	163	38	201
	19-Dec	145	36	181
Yumeshiho	17-Oct	185	43	228
	08-Nov	175	41	216
	29-Nov	161	40	201
	19-Dec	145	36	181
Nishinokaori	17-Oct	182	46	228
	08-Nov	176	40	216
	29-Nov	161	40	201
	19-Dec	145	36	181

Fig. 3. Effect of sowing time on the date of flowering and physiological maturity, and the duration between sowing, flowering time, and physiological maturity for different wheat varieties

duration from sowing to flowering was also reduced by 16-31 days for Ayahikari and Nishinokaori, by 15-33 for Nebarigoshi, and by 14-30 for Yumeshiho under late sowing. Nebarigoshi had a longer duration from sowing to flowering for the Nov. 8 and Nov. 29 sowings, compared with the other three varieties, but the duration from flowering to maturity was shorter, and thus the maturity dates were similar to those of the other varieties. For the Oct. 17 sowing, the duration from sowing to flowering was the longest for Ayahikari followed by Yumeshiho and Nishinokaori. However, the date of maturity was more or less similar for all three varieties (only Ayahikari matured one day after other two). Nebarigoshi was not sown on Oct. 17 due to seed unavailability. The duration from flowering to maturity was shorter under the Dec. 19 sowing for all varieties.

(2) Dry matter production

The statistical analyses indicated that the effects of sowing time, variety, and N application were significant for the total dry weight at flowering and maturity, as well as the leaf area index (LAI) at flowering (Table 4). These growth indices were highest for the Nov. 8 sowing, tended to be slightly lower for early sowing (Oct. 17), and were significantly reduced for late sowing. Late sowing particularly affected the LAI. The sowing × variety interaction had a significant effect on all growth indices ($P < 0.001$), but the effect of sowing × fertilizer interaction was significant only for the LAI ($P < 0.01$) (Table 4).

(3) Grain yield and yield components

The application of 80 and 150 kg N ha^{-1} split at sowing, stem elongation, and flowering significantly increased both the grain yield and GPC ($P < 0.001$) (Table 5).

As for the yield components, the decreased grain yield under the last sowing correlated well with the reduced number of heads (Table 5). The harvest index was rather high for late sowing. With respect to the cultivars, the harvest index was lower for Nishinokaori (bread wheat cultivar) than for the other three cultivars. Again, the higher yield of Nebarigoshi and Nishinokaori was well reflected in the number of heads per area. The significant effect of increased N fertilizer rate on grain yield was highly correlated with the number of heads per area. The harvest index was also slightly increased by the N fertilizer application, although the effect was not significant.

Figure 4 shows the effect of both sowing time and N application on the grain yield and GPC. There was a general tendency for the yield to be highest under optimum sowing conditions (Nov. 8), but it more or less declined under late sowing conditions. However, the pattern was different between the Ayahikai-Yumeshiho group and the Nebarigoshi-Nishinokaori group. Yield decline from optimum sowing to late sowing tended to be less for the latter group. The highest grain yield was observed for Yumeshiho (707 g m^{-2}) at the Nov. 8 sowing under the highest N application.

(4) Grain protein content (GPC)

On average, GPC was lowest at optimum sowing (Nov 8) and significantly higher for both later and earlier sowing (Table 5). Nishinokaori and Yumeshiho, the hard wheat varieties, had GPC higher than that of one soft variety (Nebarigoshi), but similar to that of the other soft variety (Ayahikari) in this experiment. The N fertilizer application also increased GPC significantly. The sowing time × variety and sowing time × fertilizer interactions were also highly significant ($P < 0.001$).

As shown in Fig. 4, we observed a general tendency of

Table 4. Effect of N application rate (split), variety, and sowing time on dry matter production at flowering and maturity, and LAI in 2012-2013

	Dry matter at flowering (g m^{-2})		Dry matter at maturity (g m^{-2})		LAI	
Sowing						
17-Oct	860.3	b	821.8	a	2.29	a
8-Nov	1062.5	a	869.2	a	2.31	a
29-Nov	678.6	c	517.4	b	1.27	b
19-Dec	740.9	bc	557.7	b	0.88	b
Variety						
Ayahikari	674.7	b	639.5	a	1.43	a
Yumeshiho	754.7	b	630.2	a	1.51	a
Nebarigoshi	915.1	a	762.1	a	1.64	a
Nishinokaori	1010.8	a	719.3	a	2.00	a
Fertilizer						
0/0/0	726.6	b	594.6	b	1.35	b
40/20/20	871.3	a	684.7	ab	1.76	a
80/40/30	903.3	a	769.2	a	1.84	a
ANOVA						
Sowing (A)	***		**		**	
Variety (B)	***		***		***	
Fertilizer (C)	***		***		***	
A x B	***		***		***	
A x C	NA		NA		**	
B x C	NA		NA		NA	
A x B x C	NA		NA		NA	

Values are expressed on a dry matter basis.

Same letters do not differ significantly ($P<0.05$, Tukey's test).

***,**,* : significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$

increasing GPC under delayed sowing conditions. Figure 4 suggests that the GPC of Ayahikari and Yumeshiho sown at the optimum timing is amenable to fit into the quality bonus window through fertilizer application, but not under late sowing conditions where GPC exceeded the higher limit of the quality bonus, regardless of the N application rate. However, Nebarigoshi and Nishinokaori were suggested to produce GPC values within the quality bonus window even under late sowing conditions.

Discussions

1. Optimal sowing time confirmed as being early to mid-November

The yield results of the two experiments (Exps. 1 and 2; Tables 3 and 5, respectively) suggested that the optimal sowing window for wheat in Japan's Kanto region is early to mid-November, which coincides with the prevalent practice in the region. Yield was significantly lower under earlier or later sowing conditions than under these optimal sowing conditions. As shown in Figure 1, the minimum temperature dropped to nearly zero in December in these three years; consequently, plants sown before or during early to mid-November could have an opportunity to produce enough fall tillers, resulting in a higher grain yield. The yield component analysis in Exp. 2 showed significantly higher number of heads per area during the optimal sowing period (Table 5).

For plants sown in the optimal period, better germination rates, good crop establishment (Farroq et al. 2008), and a longer vegetative period can be expected, along with better root growth, as compared with late

Table 5. Effect of N application rate (split), variety, and sowing time on the grain yield, GPC, and yield components in 2012-2013

	Grain yield (g m^{-2})		GPC (%)		HI		Number of heads (m^{-2})		Number of grains ($head^{-1}$)		One thousand grain weight (g)	
Sowing												
17-Oct	363.4	b	14.8	a	0.46	c	457.8	a	20.8	b	38.0	a
8-Nov	503.4	a	12.9	b	0.58	b	465.1	a	31.6	a	34.7	b
29-Nov	337.7	b	15.1	a	0.66	a	363.8	b	32.2	a	33.1	b
19-Dec	369.9	b	15.5	a	0.67	a	394.5	b	29.0	a	33.2	b
Variety												
Ayahikari	369.6	b	14.7	a	0.61	a	351.2	b	31.8	a	36.8	a
Nebarigoshi	488.1	a	12.7	b	0.65	a	500.6	a	32.5	a	30.8	c
Nishinokaori	388.1	b	15.1	a	0.54	b	449.1	ab	24.1	b	36.9	a
Yumeshiho	359.6	b	15.4	a	0.61	a	391.2	b	28.3	ab	32.7	b
Fertilizer												
0/0/0	324.6	c	13.2	c	0.58	a	377.6	b	28.1	a	34.2	a
40/20/20	398.6	b	14.7	b	0.61	a	433.4	ab	27.3	a	34.8	a
80/40/30	463.6	a	15.9	a	0.62	a	442.5	a	31.4	a	34.7	a
ANOVA												
Sowing (A)	**		**		**		***		**		***	
Variety (B)	***		***		***		***		***		**	
Fertilizer (C)	***		***		*		NS		***		**	
A x B	***		***		NS		***		***		NS	
A x C	**		***		NS		NS		NS		NS	
B x C	NS		*		NS		NS		NS		NS	
A x B x C	NS		*		NS		NS		NS		NS	

Values are expressed on dry matter basis.

Same letters do not differ significantly ($P<0.05$, Tukey's test).

***,**,* : significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$

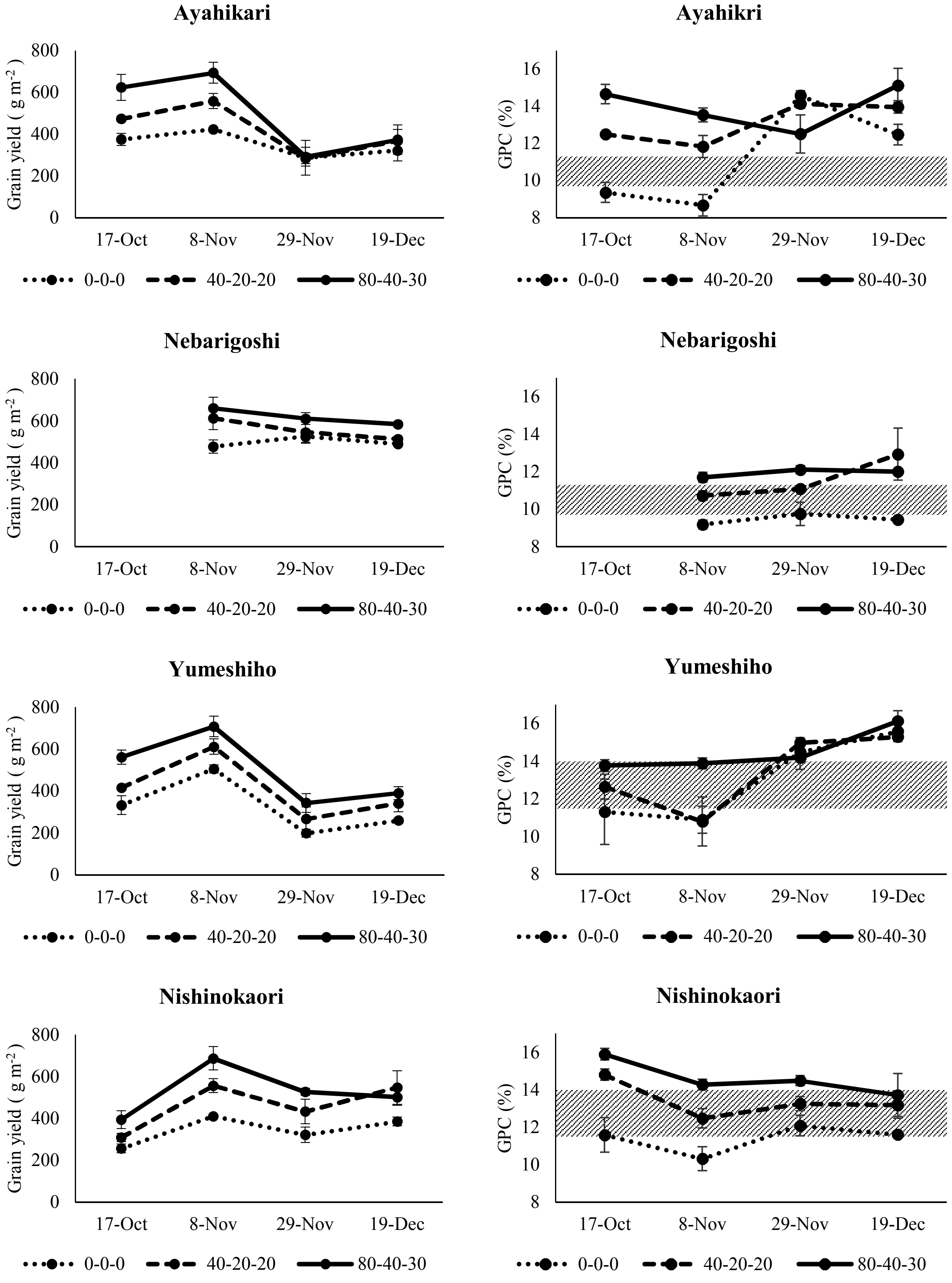


Fig. 4. Grain yield and GPC response of the soft wheat varieties (Ayahikari and Nebarigoshi), and hard wheat varieties (Yumeshiho and Nishinokaori) to different N treatments at different sowing times (2012-2013 experiment) [Different lines represent different N application rates (0-0-0, 40-20-20, and 80-40-30). Shaded areas show the quality bonus window for GPC (hard wheat: 11.5-14%; soft wheat: 9.7-11.3%). No data for Nebarigoshi in the Oct. 17 sowing group.]

sowing conditions (Barraclough & Leigh 1984). Thiry et al. (2002) also reported that optimal period sowing enhanced tillering during the fall, which is important for the final grain yield. Conversely, planting too early produces excessive tillering during the fall, which leads to competition among tillers, causing a low percentage of formed spikes (Thiry et al. 2002).

2. Delayed sowing decreased grain yield but increased GPC

We observed an increase in GPC and a decrease in grain yield under delayed sowing as a general pattern (Fig. 4). It was also reported that late sowing increases GPC, but reduces the grain yield in soils other than volcanic ash soils [e.g., Endoquolls and Eutrocryepts soils (Subedi et al. 2007)], and reduces the thousand-grain weight (Saleem et al. 2015). Barbottin et al. (2005) reported that nitrogen remobilized at maturity was highly positively correlated to nitrogen uptake at flowering. Therefore, when carbohydrate translocation is limited as a result of poor vegetative growth under late sowing, N concentration in the grain increases due to the condensation effect. The significant interaction between the sowing time and fertilizer application rate, and between sowing time and cultivar in the 2012-2013 season suggested that the effect of sowing time on the grain yield and GPC is dependent on the cultivar and fertilizer application (Table 5).

3. N application at flowering time to control GPC is only effective for optimal sowing

In this study, we observed the tendency that N application “at flowering” increased GPC, but did not significantly increase the grain yield, in both hard and soft wheat varieties grown in volcanic ash soils (Table 3). As reported in literature, most grain N comes from the pre-anthesis assimilated N (Ehdaie & Waines 2001), and N uptake continues during the grain-filling period (Asseng et al. 2002). Therefore, N application at flowering provides more N to the grain, whereas there is no more new leaf growth in the plant after flowering time (Hay and Porter 2006) and only dry matter that was translocated to the grain during the grain filling is accumulated. Therefore, N application at flowering may not add further benefit for increasing grain yield. Conversely, the N application at stem elongation tended to increase the grain yield (Table 3). The stem elongation stage is important in establishing grain yield components; therefore, providing N during this stage is effective for increasing grain yield (Kirby 1998, Hay & Porter 2006). Similar results have been reported elsewhere for the optimal period sowing conditions (Nakano & Morita 2009, Woolfolk et al. 2002, Fischer et al. 1993). Figure 5 further explains the results of Table 3, showing that the increase in grain yield was higher than the increase in GPC when N was applied at stem elongation, whereas the increase in GPC was higher than the increase in grain yield when additional N was applied at flowering time.

It has been proposed that N application at flowering can be used as a management option to adjust GPC to fit into the quality bonus window. Our results demonstrated that the application of N at flowering to increase GPC was effective only for optimal period sowing but not for late sowing, because the GPC of Kanto varieties (Ayahikari and Yumeshiho) under late sowing already exceeded the upper limit, even without N application (Figs. 2 and 4). To our knowledge, our study is the first report to discuss the effectiveness of N application at flowering time under late sowing conditions.

One of the major constraints for wheat cultivation in Japan’s Kanto region is the autumn rain, which delays the land preparation and machine sowing using drill planters. This delayed sowing causes dual economic loss to the farmers: the lower yield and GPC surpass the quality bonus window. The present results suggest little or no N application at the time of flowering, but at the stem elongation stage as a reasonable N management strategy

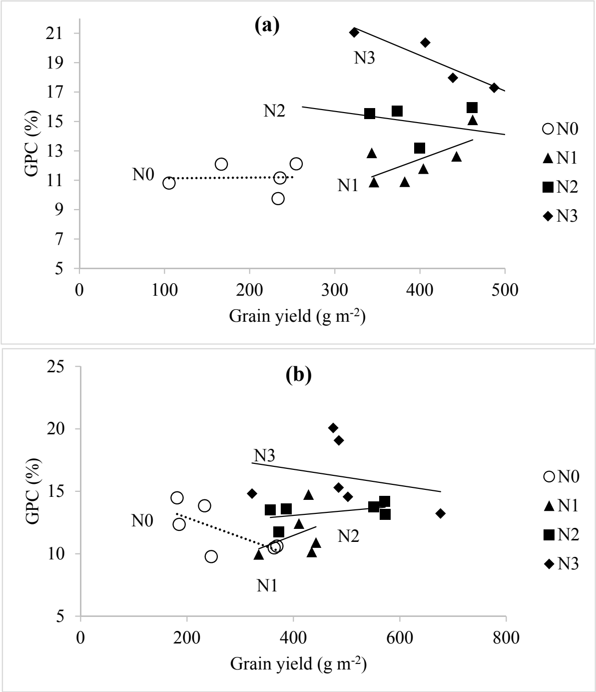


Fig. 5. Grain yield-protein relationship for different N treatments: N0: 0-0-0, N1: 60-20-0, N2: 60-20-30, N3: 60-20-60 (2010-2011 Nov. 2 sowing and 2011-2012 experiments) [(a): Yumeshiho (hard wheat]; (b): Ayahikari (soft wheat). Each data point represents a replicate.]

in case sowing is delayed.

4. Soil factors in the context of quantity of N to be applied at flowering time

According to the results, GPC of both hard and soft wheat varieties grown in volcanic ash soils had a tendency to surpass the quality bonus window with N application at flowering. When N application at flowering time was increased from 30 to 60 kg N ha^{-1}, together with high total fertilizer application before anthesis (60 and 20 kg N ha^{-1} at sowing and stem elongation, respectively), GPC increased up to 15.6 and 17.1% in soft and hard wheat, respectively (Table 3). However, according to Nakano et al. (2008), the increase of N application at flowering time from 30 to 60 kg N ha^{-1} (90 kg N ha^{-1} applied in total at sowing and tillering), the GPC of hard wheat increased only to the maximum of ca. 14.5% in Gray lowland soil. Shimazaki et al. (2015) also reported that in volcanic ash soils, the original GPC content is higher than that in Alluvial soils. These differences in GPC are suggested to be caused by different types of soil in the indigenous soil N supply. Eneji et al. (2002) reported that the native nitrogen supply capacity in volcanic ash soils is higher than that in other soils, and is further enhanced by the application of N fertilizers that simulate mineralization of the native soil nitrogen. The indigenous N content of Gray lowland soil is reported to be lower than that of volcanic ash soils (Endo et al. 2013).

In 2011-2012, GPC increased to a higher level (17.1%) compared with the other years (Table 3). The precipitation in early spring (March) when the maximum temperature starts increasing rapidly was higher in 2012 compared with the other years, and it might have enhanced the soil N supply through mineralization. These results suggested that attaining the expected GPC in par with quality bonus standards, while increasing the grain yield, is difficult. The general fertilizer recommendations based on the results of field experiments may not have merit unless the temporal variability of indigenous soil N supply is assured.

Both plant growth and grain yield were relatively higher even under no nitrogen conditions in the 2010-2011 season due to the fallowing period that preceded this experiment. The soil inorganic N content of the surface soil was in fact higher at the beginning of the experiment with no N application, but it decreased after the two years of cultivation.

5. Efficacy of N application at flowering time differs between hard and soft wheat varieties

The results showed that the effect of N application at flowering time was different between hard and soft wheat varieties. The GPC content of hard wheat tended to fit into the quality bonus window, whereas that of soft wheat surpassed it, in response to the N application at flowering time (Fig. 2). In Exp. 2 as well, it was found that 80, 40, and 30 kg N ha^{-1} at sowing, stem elongation, and flowering, respectively, resulted in the best N management strategy for the hard wheat variety Yumeshiho in attaining a high grain yield (7 tons ha^{-1}) and the standard GPC (Fig. 4). Yumeshiho is a newly developed bread wheat cultivar, and thus our results confirmed its better performance as a bread wheat cultivar grown in the volcanic ash soils in Japan's Kanto region. In contrast, the soft wheat (noodle) cultivar Ayahikari had a higher grain yield under optimal and early sowing with the same N management strategy, but resulted in excessively high GPC values that surpassed the standards (Fig. 4).

6. Adoption of cultivars from other regions that perform well under late sowing conditions

Nebarigoshi (soft wheat) and Nishinokaori (hard wheat) are the recommended cultivars for Tohoku and the western region in Japan, respectively. The yield of these cultivars was not as high as that of Yumeshiho and Ayahikari, the cultivars recommended for the Kanto region, when sown at the optimum time of the year (Fig. 4). However, these two varieties attained a higher yield than Yumeshiho and Ayahikari, and maintained their GPC within the quality bonus window, even under late sowing conditions. These results suggest the possibility of using Nebarigoshi and Nishinokaori under late sowing conditions in the Kanto region, although further investigation is needed to confirm the applicability of this strategy.

Conclusions

This study contributed to filling the information gap in previous reports regarding the interactive effects of split N fertilizer application at the flowering stage and sowing time on the grain yield and GPC of both hard and soft wheat varieties grown in volcanic ash soils.

Our results suggested that N application at the flowering time for increasing GPC is more suitable under optimum sowing conditions, where the GPC of both hard and soft wheats can be adjusted to fit into the quality bonus window. Although early to mid-November is the best time for sowing wheat in Japan's Kanto region, late sowing was also suggested as being possible with the right choice of cultivar and a proper N management strategy.

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