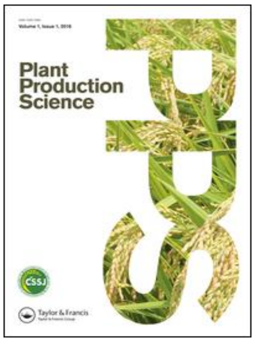

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Leaf Blade Dry Weight and Leaf Area Index × SPAD Value at Anthesis Can Be Used to Estimate Nitrogen Application Rate at Anthesis Required to Obtain Target Protein Content of Grain in Bread Wheat

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Abstract: We analyzed the relationship between growth-related traits at anthesis and protein content of grain at maturity to develop a method of estimating the nitrogen (N) application rates at anthesis required to obtain the target protein content of grain in the bread wheat (*Triticum aestivum* L.) cultivar Minaminokaori in three crop seasons in southwestern Japan. The protein content of grain had a higher positive correlation with leaf blade dry weight (DW) and the leaf area index (LAI) × SPAD value than with the SPAD value. Moreover, the relationships between leaf blade DW and protein content of grain and between LAI × SPAD value and protein content of grain could be approximated well using quadratic functions for each N application rate at anthesis. Thus, N application rate at anthesis required to obtain the target protein content of grain can be estimated by using these quadratic functions.

Key words: Anthesis, Bread wheat, Growth diagnosis, Leaf blade dry weight, Minaminokaori, Protein content of grain, *Triticum aestivum* L.

The production of bread wheat (*Triticum aestivum* L.) is increasing in Japan because of increasing demand for domestic bread wheat (Taya et al., 2003; Fujita et al., 2009). Bread wheat generally requires a high protein content of grain to make bread of acceptable quality (Wall, 1979).

Recently, a new hard red wheat cultivar Minaminokaori, which has acceptable performance as bread wheat in southwestern Japan, was developed (Fujita et al., 2009). Although Minaminokaori has a higher protein content of grain than standard cultivars when grown with standard N application method (5 g N m^{-2} at seeding, 4 g N m^{-2} at active tillering, 2 g N m^{-2} at jointing, and none at anthesis), it is difficult to obtain a sufficiently high protein content of grain for bread making (i.e., between 11.5% and 14.0%, the required range for bread wheat in Japan). Iwabuchi et al. (2007) reported that when 4 and 2 g N m^{-2} were applied at active tillering and jointing, respectively, the protein content of grain of Minaminokaori increased linearly with increasing N application at anthesis at a rate of about 0.5% per 1 g N m^{-2} in southwestern Japan. Similar results were obtained by Takayama et al. (2004). We also obtained similar results in a previous study, but the protein content of grain of Minaminokaori was increased more when 0 and 2 g N m^{-2} were applied at active tillering and jointing, respectively, and less when 8 and 2 g N m^{-2} were applied at active tillering and jointing, respectively, in southwestern Japan (Nakano et al., 2008).

The protein content of grain of wheat is influenced by cultivar, N application rate and time, seeding rate, soil characteristics, and the growth environment (Karathanasis et al., 1980; Rao et al., 1993; Geleta et al., 2002). In southwestern Japan, the root activity of wheat plants is decreased by excess moisture due to relatively high rainfall during growth, the amount of N uptake from soil by wheat plants is less than in other regions of Japan (Taya, 2001). Hence, wheat plants may translocate much more N in the leaf blades to the grains during ripening in southwestern Japan than in other regions in Japan. The protein content of grain of bread wheat often differs among fields in southwestern Japan (Tasaka and Sasaki, 2005). The reduced N uptake causes reduced protein content of grain, leading to problems in bread making. Thus, it is necessary to define a method for determining the optimal N application rate at anthesis by diagnosing the nutrient status of plants at anthesis.

The protein content of grain of wheat is influenced by leaf and stem N content during ripening. In rice, there was a positive correlation between leaf color value and N

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Abbreviations : DW, dry weight; LAI, leaf area index; N, nitrogen; 0–0N, 0 and 0 g N m^{-2} applied at active tillering and jointing, respectively; 4–2N, 4 and 2 g N m^{-2} applied at active tillering and jointing, respectively.

contents of leaf and stem (Tanno et al., 1982; Kumagai et al., 1991). In wheat, there was a similar positive correlation between leaf color value and N contents of whole-plant and leaf (Matsunaka et al., 1997; Sato, 2000; Takebe et al., 2006). The leaf blade SPAD value is closely related to the chlorophyll content of leaf (Yadava, 1986; Campbell et al., 1990; Schaper and Chacko, 1991). Takebe et al. (2006) found that SPAD value at full heading can be used to estimate N application rate at full heading required to obtain target protein content of grain of the bread wheat cultivar Kitanokaori in Hokkaido. However, Spaner et al. (2005) indicated that it may be difficult to prescribe the optimal N application rate based on solely SPAD measurements, since the critical SPAD value may vary among years, locations, cultivars, and soil characteristics. Akaiwa et al. (2007) reported that the leaf area index (LAI) × SPAD value might represent the total amount of chlorophyll.

Minaminokaori has slightly low grain yield as a result of the small number of spikes and small number of grains per spike compared with a standard cultivar (Fujita et al., 2009). Increasing grain yield together with protein content of grain is an important goal in wheat production (Sarandon, 1999). Kramer (1979) and Löffler et al. (1985) indicated that there was an inverse relationship between grain yield and protein content of wheat.

The objectives of the present study were to analyze the relationships between growth-related traits at anthesis and N application rates at anthesis required to obtain the target protein content of grain, and to develop a practical method for determining optimal N application rate at anthesis by diagnosing the nutrient status at anthesis in the bread wheat cultivar Minaminokaori in southwestern Japan. In addition, we analyzed the relationships between growth-related traits at anthesis and grain yield at maturity to determine the traits at anthesis related to grain yield.

Materials and Methods

1. Crop management

The study was conducted during the 2003–2004, 2004–2005, and 2008–2009 crop seasons on a Lowland Paddy soil at the National Agricultural Research Center for Kyushu Okinawa Region (lat. 33° 12′ N, long. 130° 30′ E, 10 m asl) in Chikugo, Fukuoka, Japan. The experimental details for the 2003–2004 and 2004–2005 crop seasons were described by Nakano et al. (2008) and Nakano and Morita (2009), respectively.

In the 2003–2004 crop season, the experiment had a 3 (N application rate at active tillering) × 4 (N application rate at anthesis) factorial design, arranged in a randomized complete block split-plot with three replicates. The main plot and subplot factors were N application rate at active tillering and N application rate at anthesis, respectively. The field received 5.0 g N m^{-2}, 2.2 g P m^{-2}, and 4.2 g K m^{-2} in the form of chemical fertilizer broadcast by hand 1 day before seeding, and the fertilizer was incorporated into the soil by plowing. The plots were sown on 18 November 2003 at 175 seeds m^{-2} with mechanical seeder. Plants received 0, 4, or 8 g N m^{-2} in the form of ammonium sulfate at active tillering (late January), 2 g N m^{-2} at jointing (late February), and 0, 2, 4, or 6 g N m^{-2} at anthesis (mid-April). These fertilizers were broadcast by hand on the plot surface and were not incorporated into the soil. After trimming, each plot was 2.8 m wide × 5.0 m long, with two ridges per plot (each containing four rows spaced 0.3 m apart).

In the 2004–2005 crop season, the experiment had a 2 (seeding rate) × 2 (N application rate at active tillering and jointing) × 4 (N application rate at anthesis) factorial design, arranged in a randomized complete block split-split-plot with three replicates. The main plot, subplot, and sub-subplot factors were seeding rate, N application rate at active tillering and jointing, and N application rate at anthesis, respectively. The field received 5.0 g N m^{-2}, 2.2 g P m^{-2}, and 4.2 g K m^{-2} in the form of chemical fertilizer broadcast by hand 1 day before seeding, and the fertilizer was incorporated into the soil by plowing. The plots were hand-sown on 17 November 2004 at 50 or 150 seeds m^{-2}. Plants received 0 or 4 g N m^{-2} in the form of ammonium sulfate at active tillering (late January), 0 or 2 g N m^{-2} at jointing (late February), and 0, 2, 4, or 6 g N m^{-2} at anthesis (mid-April). These fertilizers were broadcast by hand on the plot surface and were not incorporated into the soil. After trimming, each plot was 2.8 m wide × 5.0 m long, with two ridges per plot (each containing four rows spaced 0.3 m apart).

In the 2008–2009 crop season, the experiment had a 3 (N application time before heading) × 4 (N application rate at anthesis) factorial design, arranged in a randomized complete block split-plot with three replicates. The main plot and subplot factors were N application time before heading and N application rate at anthesis, respectively. The field received 5.4 g N m^{-2}, 2.4 g P m^{-2}, and 4.5 g K m^{-2} in the form of chemical fertilizer broadcast by hand 1 day before seeding, and the fertilizer was incorporated into the soil by plowing. The plots were sown on 20 November 2008 at 7 g m^{-2} of seed with mechanical seeder. Plants received 4 g N m^{-2} in the form of ammonium sulfate at active tillering (early February), jointing (late February), or late jointing (mid-March) and 0, 2, 4, or 6 g N m^{-2} at anthesis (mid-April). These fertilizers were broadcast by hand on the plot surface and were not incorporated into the soil. After trimming, each plot was 2.25 m wide × 3.5 m long, with one ridge per plot (each containing four rows spaced 0.3 m apart).

2. Growth and yield survey

At anthesis, plants in pre-marked sample areas (0.7 m

Table 1. Effects of N application rate at active tillering and N application rate at anthesis on grain yield and protein content at maturity and growth-related traits at anthesis of the bread wheat cultivar Minaminokaori in 2003–2004 crop season.

		Maturity					Anthesis				
		Grain yield ($g\ m^{-2}$)	Number of Spikes (m^{-2})	Number of Grains ($spike^{-1}$)	1000-grain weight (g)	Protein content of grain (%)	Whole-plant DW ($g\ m^{-2}$)	Leaf blade DW ($g\ m^{-2}$)	Number of spikes (m^{-2})	LAI	SPAD value
N application rate at active tillering ($g\ N\ m^{-2}$) (A)†											
0		432b‡	380c	30.3	37.7	11.7 c	820	116 c	390 b	2.74 b	42.6 c
4		500a	432b	31.0	37.3	12.6 b	857	141 b	440 ab	3.41 ab	45.8 b
8		528a	464a	32.1	35.4	13.7 a	872	160 a	465 a	3.82 a	47.9 a
N application rate at anthesis ($g\ N\ m^{-2}$) (B)†											
0		480	429	30.6	36.5	11.0 d					
2		479	419	31.0	37.0	12.2 c					
4		488	425	31.3	36.8	13.4 b					
6		500	428	31.7	36.9	14.1 a					
A×B											
0	0	430	388	29.9	37.1	9.7 a					
	2	417	360	30.6	38.1	10.9 b					
	4	427	379	29.6	38.2	12.4 c					
	6	455	392	31.0	37.6	13.7 d					
4	0	491	431	30.8	36.9	10.9 a					
	2	490	437	29.9	37.5	12.1 b					
	4	506	433	31.6	37.1	13.4 c					
	6	512	428	31.8	37.6	14.0 d					
8	0	519	468	31.2	35.6	12.5 a					
	2	530	460	32.4	35.5	13.6 b					
	4	532	463	32.7	35.1	14.2 c					
	6	533	465	32.3	35.5	14.5 c					
ANOVA											
N application rate at active tillering ($g\ N\ m^{-2}$) (A)		**	**	NS§	NS	**	NS	**	*	**	**
N application rate at anthesis ($g\ N\ m^{-2}$) (B)		NS	NS	NS	NS	**					
A×B		NS	NS	NS	NS	**					

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† Values represent the means of the sub-plots.
‡ Means within a treatment that are followed by the same letters do not differ significantly ($P<0.05$, LSD).
§ NS, not significant.

wide×1.0 m long, 1.5 m wide×1.0 m long, and 1.5 m wide×1.0 m long in the 2003–2004, 2004–2005, and 2008–2009 crop seasons, respectively) were harvested and the number of spikes was counted. The chlorophyll content index (the SPAD value) of the flag leaf was measured with a chlorophyll meter (SPAD 502, Minolta Co. Ltd., Osaka, Japan). About 10% of the plants randomly selected based on the number of spikes from main and tiller stems were divided into leaf blades (not including dead leaf blade), dead leaf blades, leaf sheaths plus stems, and spikes. After measurement of the area of leaf blades with a leaf area meter (LI-3050A/4, LI-COR Ltd., Lincoln, Nebraska, USA), each plant part was dried at 80ºC for 2 days in a ventilated oven to determine their dry weights (DW). The DW of the remaining plants was also determined in the same way.

At maturity, plants in pre-marked sample areas (1.4 m wide×3.0 m long, 1.4 m wide×3.0 m long, and 1.5 m wide×2.0 m long in the 2003–2004, 2004–2005, and 2008–2009 crop seasons, respectively) were harvested and were air-dried to a constant weight. The number of spikes was counted, and then air-dried plants were threshed to determine their grain weight. The number of grains making up 20 g was counted with an electronic seed counter (Fujimoto Science Company Ltd., Tokyo, Japan), and the 1000-grain weight was calculated from this value. The number of grains per spike was calculated as (grain weight×1000)/(number of spikes×1000-grain weight). Grain yield and the 1000-grain weight were corrected to a 12.5% moisture basis. Grain was ground to pass through a 0.75-mm Retsch Grinding Mill (2M1, Retsch GmbH & Co. KG, Haan, Germany). These samples were used to determine the protein content of grain by means of the Kjeldahl method (N×5.70). The protein content of grain was corrected to a 13.5% moisture basis.

3. Statistical analysis

Differences among treatments were tested by means of analysis of variance (ANOVA). When an *F*-test was significant ($P<0.05$), we used the least-significant difference (LSD) test to identify significant ($P<0.05$) differences between individual treatments.

Results

1. Effects of N application rate at active tillering and N application rate at anthesis on grain yield and protein content at maturity and growth-related traits at anthesis

The grain yield increased with increasing N application rate at active tillering (Table 1). The high grain yield with application of 8 g N m^{-2} at active tillering resulted from an increased number of spikes. The protein content of grain increased from 11.7% to 13.7% with increasing N application rate from 0 to 8 g N m^{-2} at active tillering. It increased from 11.0% to 14.1% with increasing N application rate from 0 to 6 g N m^{-2} at anthesis. However, with 8 g N m^{-2} applied at active tillering, the protein content of grain did not differ between applications of 4 and 6 g N m^{-2} at anthesis.

Among growth related traits at anthesis, the leaf blade DW, the number of spikes, the LAI, and the SPAD value increased with increasing N application rate at active tillering (Table 1).

2. Effects of seeding rate, N application rate at active tillering and jointing, and N application rate at anthesis on grain yield and protein content at maturity and growth-related traits at anthesis

The grain yield was higher when 4 and 2 g N m^{-2} were applied at active tillering and jointing, respectively (4–2N), than when 0 and 0 g N m^{-2} were applied (0–0N) (Table 2). The high grain yield with 4–2N resulted from an increased number of spikes and an increased number of grains per spike. The protein content of grain was higher with 4–2N (11.5%) than with 0–0N (10.8%). It increased from 9.4% to 12.9% with increasing N application rate from 0 to 6 g N m^{-2} at anthesis.

Among growth related traits at anthesis, the whole-plant DW and leaf blade DW were higher with 4–2N than with 0–0N (Table 2). The number of spikes was greater with 150 seeds m^{-2} than with 50 seeds m^{-2}. It was greater with 4–2N than with 0–0N. The LAI was higher with 4–2N than with 0–0N. The SPAD value was lower with 150 seed m^{-2} than with 50 seed m^{-2}. It was higher with 4–2N than with 0–0N.

3. Effects of N application time before heading and N application rate at anthesis on grain yield and protein content at maturity and growth-related traits at anthesis

The grain yield was higher with N application at jointing than with N applications at active tillering and late jointing (Table 3). The high grain yield with N application at jointing resulted from an increased number of spikes and a maintained number of grains per spikes. The grain yield increased with increasing N application rate at anthesis. The high grain yield with application of 6 g N m^{-2} at anthesis resulted from an increased 1000-grain weight. The protein content of grain was lower with N application at jointing (11.1%) than with N applications at active tillering (11.5%) and late jointing (11.7%). It increased from 9.7% to 13.1% with increasing N application rate from 0 to 6 g N m^{-2} at anthesis. However, with 4 and 6 g N m^{-2} applied at anthesis, the protein content of grain did not differ among N application times before heading.

Among growth related traits at anthesis, the number of spikes was greatest with N application at active tillering, followed by N applications at jointing and late jointing (Table 3).

Table 2. Effects of seeding rate, N application rate at active tillering and jointing, and N application rate at anthesis on grain yield and protein content at maturity and growth-related traits at anthesis of the bread wheat cultivar Minaminokaori in 2004–2005 crop season.

	Maturity					Anthesis				
	Grain yield ($g\ m^{-2}$)	Number of Spikes (m^{-2})	Number of Grains ($spike^{-1}$)	1000-grain weight (g)	Protein content of grain (%)	Whole-plant DW ($g\ m^{-2}$)	Leaf blade DW ($g\ m^{-2}$)	Number of spikes (m^{-2})	LAI	SPAD value
Seeding rate (seeds m^{-2}) (A)†										
50	385	232 b¶	42.5 a	38.7	11.2	646	90	248 b	2.18	40.6 a
150	375	309 a	31.7 b	37.6	11.1	744	88	331 a	2.19	37.2 b
N application rate at active tillering and jointing (B)†										
0–0N‡	278 b	221 b	34.2 b	37.9	10.8 b	650 b	69 b	247 b	1.60 b	35.3 b
4–2N§	482 a	320 a	40.0 a	38.3	11.5 a	739 a	109 a	331 a	2.77 a	42.5 a
N application rate at anthesis ($g\ N\ m^{-2}$) (C)†										
0	366	274	36.4	36.8 c	9.4 d					
2	379	275	36.8	38.1 b	10.6 c					
4	389	268	38.0	38.4 ab	11.7 b					
6	385	264	37.3	39.1 a	12.9 a					
ANOVA										
Seeding rate (A)	NS#	*	*	NS	NS	NS	NS	*	NS	*
N application rate at active tillering and jointing (B)	**	**	**	NS	*	**	**	**	**	**
N application rate at anthesis (C)	NS	NS	NS	*	**					
A×B	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
A×C	NS	NS	NS	NS	NS					
B×C	NS	NS	NS	NS	NS					
A×B×C	NS	NS	NS	NS	NS					

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† Values represent the means of the sub-plots.
‡ 0 and 0 $g\ N\ m^{-2}$ applied at active tillering and jointing, respectively.
§ 4 and 2 $g\ N\ m^{-2}$ applied at active tillering and jointing, respectively.
¶ Means within a treatment that are followed by the same letters do not differ significantly ($P<0.05$, LSD).
# NS, not significant.

Table 3. Effects of N application time before heading and N application rate at anthesis on grain yield and protein content at maturity and growth-related traits at anthesis of the bread wheat cultivar Minaminokaori in 2008–2009 crop season.

		Maturity					Anthesis				
		Grain yield ($g\ m^{-2}$)	Number of Spikes (m^{-2})	Number of Grains ($spike^{-1}$)	1000-grain weight (g)	Protein content of grain (%)	Whole-plant DW ($g\ m^{-2}$)	Leaf blade DW ($g\ m^{-2}$)	Number of spikes (m^{-2})	LAI	SPAD value
N application time before heading (A)†											
Active tillering		485 b‡	406 a	27.0 c	44.2	11.5 a	959	121	440 a	3.17	40.3
Jointing		564 a	381 a	33.9 b	43.7	11.1 b	853	107	368 b	2.82	41.6
Late jointing		484 b	307 b	35.8 a	44.1	11.7 a	847	108	301 c	2.78	43.6
N application rate at anthesis ($g\ N\ m^{-2}$) (B)†											
0		484 b	353	31.9	43.5 b	9.7 d					
2		514 ab	362	32.6	44.2 ab	10.8 c					
4		516 ab	365	32.7	44.0 ab	12.1 b					
6		530 a	378	31.9	44.3 a	13.1 a					
A×B											
Active tillering	0	462	395	26.8	43.6	9.7 ab					
	2	472	397	26.7	44.4	10.8 ab					
	4	504	424	26.9	44.2	12.4					
	6	501	406	27.7	44.5	13.3					
Jointing	0	525	367	33.2	43.0	9.3 b					
	2	578	386	34.4	43.8	10.4 b					
	4	562	368	34.6	44.1	11.8					
	6	590	404	33.4	43.8	12.9					
Late jointing	0	464	298	35.6	43.8	10.0 a					
	2	493	304	36.6	44.3	11.2 a					
	4	480	302	36.5	43.7	12.2					
	6	499	324	34.5	44.6	13.3					
ANOVA											
N application time before heading (A)		*	**	**	NS§	*	NS	NS	**	NS	NS
N application rate at anthesis ($g\ N\ m^{-2}$) (B)		**	NS	NS	**	**					
A×B		NS	NS	NS	NS	*					

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† Values represent the means of the sub-plots.
‡ Means within a treatment that are followed by the same letters do not differ significantly ($P<0.05$, LSD).
§ NS, not significant.

Table 4. Correlation coefficients between growth-related traits at anthesis and grain yield of the bread wheat cultivar Minaminokaori at each N application rate at anthesis.†

Growth-related traits at anthesis	N application rate at anthesis (g N m^{-2})			
	0	2	4	6
Whole-plant DW	0.733*	0.707*	0.714*	0.741*
Leaf blade DW	0.832**	0.748*	0.787**	0.763*
Number of spikes	0.713*	0.632*	0.665*	0.686*
LAI	0.901***	0.832**	0.870**	0.852**
SPAD value	0.847**	0.779**	0.807**	0.774**
Leaf blade DW×Number of spikes	0.747*	0.663*	0.700*	0.687*
Leaf blade DW×LAI	0.804**	0.730*	0.764*	0.734*
Leaf blade DW×SPAD value	0.815**	0.736*	0.768**	0.738*
Number of spikes×LAI	0.787**	0.710*	0.749*	0.739*
Number of spikes×SPAD value	0.790**	0.707*	0.738*	0.741*
LAI×SPAD value	0.878***	0.810**	0.841**	0.816**

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Data from the 2003–2004, 2004–2005, and 2008–2009 crop seasons were included in the value.

Table 5. Correlation coefficients between grain yield components and LAI at anthesis of the bread wheat cultivar Minaminokaori at each N application rate at anthesis. †

Grain yield components	N application rate at anthesis (g N m^{-2})			
	0	2	4	6
Number of spikes per m^2	0.909***	0.893***	0.902***	0.904***
Number of grains per spike	–0.172	–0.226	–0.243	–0.170
Number of grains per m^2	0.902***	0.888***	0.935***	0.918***
1000-grain weight	0.098	–0.014	0.020	–0.030

*** Significant at the 0.001 probability level.
† Data from the 2003–2004, 2004–2005, and 2008–2009 crop seasons were included in the value.

4. Correlation coefficients between growth-related traits at anthesis and grain yield at maturity for each N application rate at anthesis

We analyzed the relationships between growth-related traits at anthesis and grain yield at maturity by using the data of three crop seasons (Tables 1–4). With 0 g N m^{-2} applied at anthesis, the grain yield was significantly ($P<0.001$) and positively correlated with the LAI and the LAI×SPAD value (Table 4). At 2 g N m^{-2}, it was significantly ($P<0.01$) and positively correlated with the LAI, the SPAD value, and the LAI×SPAD value. At 4 g N m^{-2}, it was significantly ($P<0.01$) and positively correlated with the leaf blade DW, the LAI, the SPAD value, the leaf blade DW×SPAD value, and the LAI×SPAD value. At 6 g N m^{-2}, it was significantly ($P<0.01$) and positively correlated with the LAI, the SPAD value, and the LAI×SPAD value.

The LAI was significantly ($P<0.001$) positively correlated with the number of spikes and the number of grains per m^2 (Table 5).

5. Correlation coefficients between growth-related traits at anthesis and protein content of grain at maturity for each N application rate at anthesis

We analyzed the relationships between growth-related traits at anthesis and protein content of grain at maturity by using the data of three crop seasons (Tables 1–3, 6). With 0 g N m^{-2} applied at anthesis, the protein content of grain was significantly ($P<0.001$) and positively correlated with the leaf blade DW, the leaf blade DW×LAI, the leaf blade DW×SPAD value, and the LAI×SPAD value (Table 6). At 2 g N m^{-2}, it was significantly ($P<0.001$) and positively correlated with the leaf blade DW×SPAD value. At 4 g N m^{-2}, it was significantly ($P<0.001$) and positively correlated with the leaf blade DW, the LAI, the leaf blade DW×number of spikes, the leaf blade DW×LAI, the leaf blade DW×SPAD value, the number of spikes×LAI, the number of spikes×SPAD value, and the LAI×SPAD value. At 6 g N m^{-2}, it was significantly ($P<0.001$) and positively correlated with the leaf blade DW, the LAI, the SPAD

Table 6. Correlation coefficients between growth-related traits at anthesis and protein content of grain of the bread wheat cultivar Minaminokaori at each N application rate at anthesis. †

Growth-related traits at anthesis	N application rate at anthesis (g N m^{-2})			
	0	2	4	6
Whole-plant DW	0.464	0.413	0.584*	0.580
Leaf blade DW	0.896***	0.834**	0.940***	0.951***
Number of spikes	0.670*	0.631	0.799**	0.778**
LAI	0.827**	0.747*	0.871***	0.880***
SPAD value	0.842**	0.758*	0.828**	0.885***
Leaf blade DW×Number of spikes	0.854**	0.813**	0.932***	0.913***
Leaf blade DW×LAI	0.919***	0.868**	0.955***	0.942***
Leaf blade DW×SPAD value	0.930***	0.873***	0.954***	0.965***
Number of spikes×LAI	0.814**	0.763*	0.895***	0.873***
Number of spikes×SPAD value	0.812**	0.765**	0.904***	0.896***
LAI×SPAD value	0.890***	0.819**	0.915***	0.923***

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Data from the 2003–2004, 2004–2005, and 2008–2009 crop seasons were included in the value.

value, the leaf blade DW×number of spikes, the leaf blade DW×LAI, the leaf blade DW×SPAD value, the number of spikes×LAI, the number of spikes×SPAD value, and the LAI×SPAD value.

6. Relationships between leaf blade DW or LAI × SPAD value at anthesis and protein content of grain at maturity for each N application rate at anthesis

The relationships between leaf blade DW and protein content of grain and between the LAI×SPAD value and protein content of grain were well approximated by using quadratic functions for each N application rate at anthesis (Fig. 1).

Discussion

We analyzed the relationships between growth-related traits at anthesis and grain yield at maturity to determine the traits at anthesis related to grain yield in the bread wheat cultivar Minaminokaori by using the data of three crop seasons in southwestern Japan (Tables 1–5). Minaminokaori has slightly low grain yield as a result of the small number of spikes and small number of grains per spike compared with a standard cultivar (Fujita et al., 2009). The grain yield slightly increased with the increase of N application rate at anthesis (Tables 1–3). Fukushima et al. (2003) have reported a positive correlation between the LAI at anthesis and grain yield at maturity for the wheat cultivars Iwainodaichi and Chikugoizumi. In the present study, we confirmed this result for Minaminokaori (Table 4). The LAI at anthesis had a high positive correlation with the number of spikes and the number of grains per m^2 (Table 5) A similar result was obtained by Frederick and Bauer (1999), who found that the LAI at booting, whole-plant DW near anthesis, and the number of grains per m^2 at maturity are highly correlated with one another in soft red winter wheat. The LAI after anthesis is generally important to produce photosynthate to fill grains in wheat. Thus, high LAI at anthesis can improve grain yield by increasing the number of grains per m^2.

We analyzed the relationship between growth-related traits at anthesis and protein content of grain at maturity to develop a method for estimating the N application rates at anthesis required to obtain the target protein content of grain in the bread wheat cultivar Minaminokaori by using the data of three crop seasons in southwestern Japan (Tables 1–3, 6, Fig. 1). Many scientists have reported a significant interaction between protein content of grain at maturity and the leaf blade SPAD value before heading or at anthesis in wheat (Matsunaka et al., 1997; Sato, 2000; Fukushima et al., 2004; López-Bellido et al., 2004; Spaner et al., 2005; Takebe et al., 2006; Poblaciones et al., 2009). In the present study, we confirmed this result, but found that the protein content of grain had a higher positive correlation with leaf blade DW and the LAI×SPAD value than with the SPAD value (Table 6). The leaf SPAD value is closely related to the chlorophyll content of leaf (Yadava, 1986; Campbell et al., 1990; Schaper and Chacko, 1991). However, leaf blade DW and the LAI×SPAD value may better represent the total amount of chlorophyll. Spaner et al. (2005) indicated that it may be difficult to prescribe the optimal N application rate based on solely SPAD measurements, since the critical SPAD value may vary among years, locations, cultivars, and soil characteristics. Kramer (1979) and Löffler et al. (1985) reported an inverse relationship between grain yield and protein content of wheat. In the present study, leaf blade DW had a

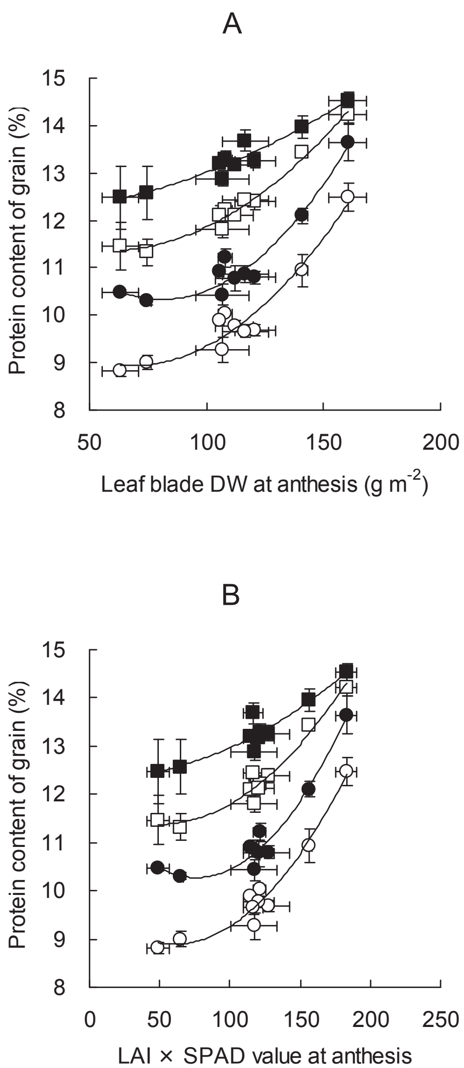

Fig. 1. Relationships between leaf blade DW (A) or LAI × SPAD value (B) at anthesis and protein content of grain of the bread wheat cultivar Minaminokaori for each N application rate at anthesis.

N application rates at anthesis were ○, 0 g N m^{-2}; ●, 2 g N m^{-2}; □, 4 g N m^{-2}; and ■, 6 g N m^{-2}. Values represent the means ± SE of the results from three replicates.

A: 0 g N m^{-2}, $y=0.0004x^2-0.0536x+10.742$, $R^2=0.936$; 2 g N m^{-2}, $y=0.0005x^2-0.0831x+13.704$, $R^2=0.942$; 4 g N m^{-2}, $y=0.0003x^2-0.0316x+12.258$, $R^2=0.978$; 6 g N m^{-2}, $y=0.0001x^2-0.0018x+12.153$, $R^2=0.930$.

B: 0 g N m^{-2}, $y=0.0002x^2-0.028x+9.7329$, $R^2=0.958$; 2 g N m^{-2}, $y=0.0003x^2-0.0443x+11.957$, $R^2=0.960$; 4 g N m^{-2}, $y=0.0002x^2-0.0166x+11.794$, $R^2=0.961$; 6 g N m^{-2}, $y=0.00007x^2-0.0006x+12.338$, $R^2=0.895$.

high positive correlation with the SPAD value×LAI (data not shown). The grain yield was strongly influenced by the LAI at anthesis (Table 4). Thus, the traits affecting grain yield as well as those related to chlorophyll content of leaf blade might be effective to diagnose the nutrient status at anthesis. Based on the present results, leaf blade DW and the LAI×SPAD value may be more suitable for use in growth diagnosis. Moreover, the relationships between leaf blade DW and protein content of grain and between the LAI×SPAD value and protein content of grain could be approximated well using quadratic functions for each N application rate at anthesis (Fig. 1). Thus, N application rate at anthesis required to obtain the target protein content of grain can be estimated by using these quadratic functions. Although the same measurement of the LAI×SPAD value as those conducted in the present study are difficult in producing area, the LAI can be measured by plant canopy analyzer (Welles and Norman, 1991) and the LAI×SPAD value may be measured by remote sensing of the normalized difference vegetation index (Akaiwa et al., 2007). The protein content of grain of wheat is influenced by cultivar, N application rate and time, seeding rate, soil characteristics, and the growth environment (Karathanasis et al., 1980; Rao et al., 1993; Geleta et al., 2002). Takahashi and Anwar (2008) indicated that the effectiveness of N application in wheat was influenced by soil characteristics. This method was obtained by the study conducted during the 2003–2004, 2004–2005, and 2008–2009 crop seasons on a Gray Lowland soil in Chikugo. Thus, regional trials of this method in various sites are needed.

Farmers are under pressure to reduce their production costs. We previously reported that N application at anthesis is more effective than application before heading for increasing the protein content of grain, but that N application before heading is more effective than application at anthesis for increasing the grain yield (Nakano et al., 2008; Nakano and Morita, 2009). Based on these results and the results of the present study, farmers should apply the minimum N application rate at anthesis required to obtain the target protein content of grain. This rate can be determined by means of growth diagnosis at anthesis, as described in the present paper.

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* In Japanese with English abstract.
** In Japanese with English summary.
*** In Japanese with English title.