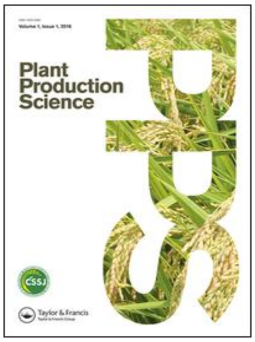


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# Effects of Seeding Rate and Nitrogen Application Rate on Grain Yield and Protein Content of the Bread Wheat Cultivar 'Minaminokaori' in Southwestern Japan

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**Abstract** : We examined the effects of seeding rate, 50 or 150 seeds m$^{-2}$, nitrogen (N) application rate at active tillering and jointing, 4 and 2 g N m$^{-2}$, respectively, or none, and N application rate at anthesis, 0, 2, 4, or 6 g N m$^{-2}$, on grain yield and protein content of a bread wheat cultivar, 'Minaminokaori', during the 2004–2005 crop season in southwestern Japan. Grain yield was similar at a seeding rate of 50 and 150 seeds m$^{-2}$. It was higher when 4 and 2 g N m$^{-2}$ were applied at active tillering and jointing, respectively (4–2N), than when no N was applied at these stages (0–0N). However, it was not influenced by N application rate at anthesis. Grain protein content was similar at 50 and 150 seeds m$^{-2}$. It was higher in 4–2N than in 0–0N. It was the highest when 6 g N m$^{-2}$ was applied at anthesis, followed by 4, 2, and 0 g N m$^{-2}$. The SPAD value at anthesis was higher at 50 than 150 seeds m$^{-2}$, but leaf area index (LAI) at anthesis was similar at 50 and 150 seeds m$^{-2}$ and protein content of grain was nearly the same at 50 and 150 seeds m$^{-2}$ irrespective of N application rate at anthesis. LAI and the SPAD value were higher in 4–2N than in 0–0N and the protein content of grain was also higher in 4–2N than in 0–0N irrespective of N application rate at anthesis. Therefore, both LAI and the SPAD value may be important traits related to the N application rate at anthesis suitable for yielding wheat grain with a high protein content.

**Key words** : Anthesis, Bread wheat, Grain protein content, Grain yield, 'Minaminokaori', Nitrogen application rate, Seeding rate, *Triticum aestivum* L.

The production of bread wheat is increasing in Japan owing to increasing demand for bread flour made from locally grown wheat (Taya et al., 2003). Unlike noodles, wheat with a high protein content of grain is needed to make bread (Wall, 1979; Takayama et al., 2004; Iwabuchi et al., 2007). Recently, a new hard red wheat cultivar, 'Minaminokaori', that has acceptable traits as bread wheat in southwestern Japan was developed by the National Agricultural Research Center for Kyushu Okinawa Region (Seki et al., 2005). Although 'Minaminokaori' has a higher protein content of grain than standard cultivars when grown with standard nitrogen (N) application rates (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 still difficult to obtain a sufficiently high protein content of grain for bread making (i.e., between 11.5% and 14.0%, a reference value for bread wheat in Japan). Because southwestern Japan has a short growing season owing to its warm climate and relatively high precipitation, less N is taken up by wheat plants during their growth than in other regions of Japan (Taya, 2001).

Protein content of grain generally differs among fields in southwestern Japan. This causes problems in bread making. Recently, we reported that N application at active tillering is more effective than that at anthesis for increasing the grain yield of 'Minaminokaori' in southwestern Japan, but that N application at anthesis is more effective than that at active tillering for increasing protein content of grain (Nakano et al., 2008). 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' and 'Nishinokaori' increased linearly with increasing N application at anthesis at a rate of about 0.5% per 1 g N m$^{-2}$ in southwestern Japan. Takayama et al. (2004) reported a similar result in western Japan. We 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). Therefore, we must investigate how the N application rate at anthesis necessary for a sufficiently high protein content of grain is determined.

The effects of seeding rate on grain yield and protein content have long been studied. Kiesselbach and Sprague (1926), Tompkins et al. (1991), Geleta

Received 29 November 2007. Accepted 15 May 2008. Corresponding author: H. Nakano (nakanohr@affrc.go.jp, fax +81-942-53-7776).
**Abbreviations :** LAI, leaf area index; SPAD, chlorophyll meter; 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

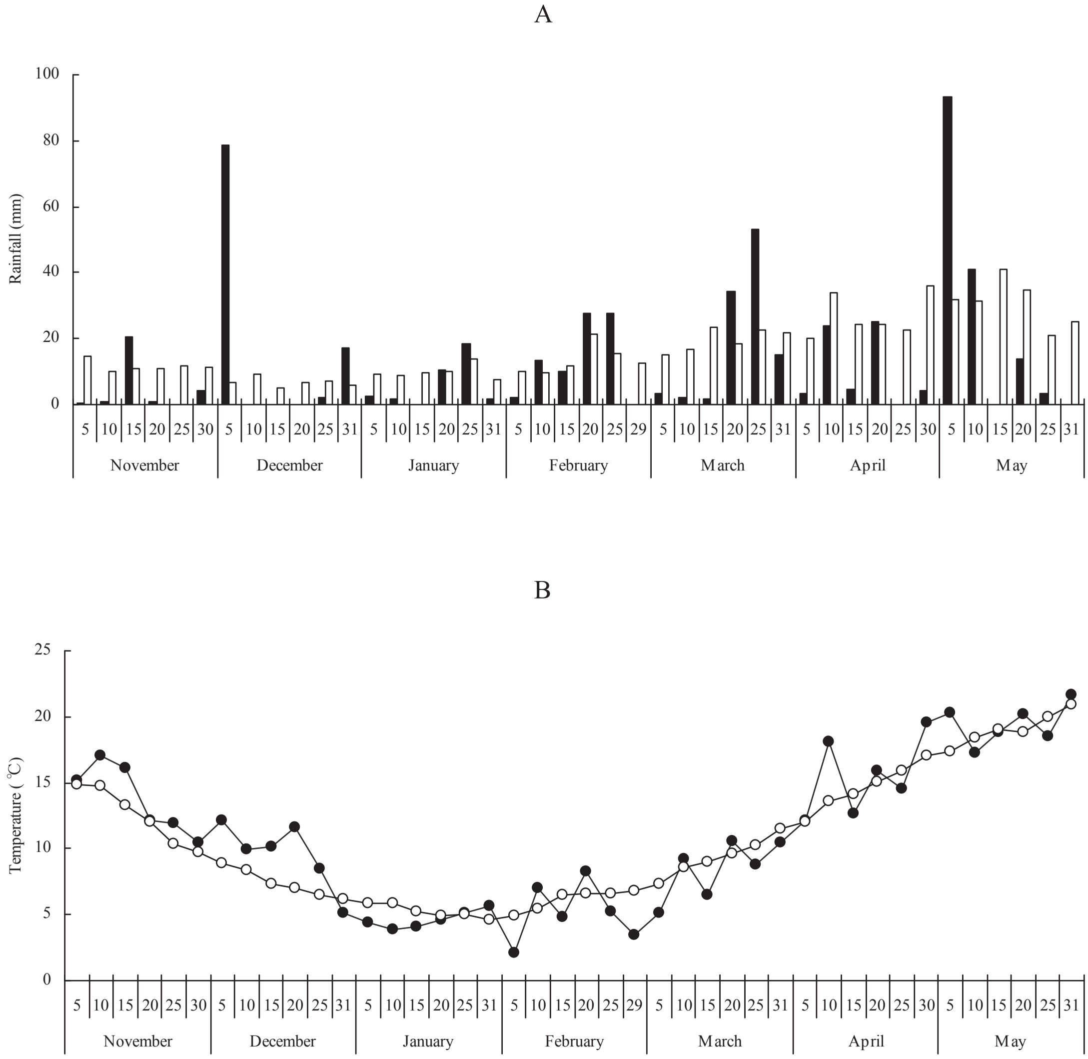


Fig. 1. Rainfall (A) and mean temperature (B) at the Chikugo study site in 2004 and 2005 (black bars and points), versus 30 years (white bars and points) from 1971 to 2000.
Rainfall and mean temperature were measured using an Agricultural Meteorological Data Acquisition System (Yokogawa Denshikiki Co. Ltd., Tokyo, Japan) at the National Agricultural Research Center for Kyushu Okinawa Region, Fukuoka, Japan.

et al. (2002), and Ozturk et al. (2006) reported that grain yield increased with increasing seeding rate. However, Iwabuchi et al. (2000) and Fukushima et al. (2004) reported that grain yield of an early-seeded wheat, 'Iwainodaichi', did not increase with increasing seeding rate in southwestern Japan. Bavec et al. (2002) and Geleta et al. (2002) reported that protein content of grain decreased with increasing seeding rate. In contrast, Campbell et al. (1991) and McLeod et al. (1996) reported that protein content of grain was not influenced by seeding rate. However, information on the interactions between seeding rate and N application rates at different stages on grain yield and protein content is limited.

The objectives of the present study were to determine the effects of seeding rate, N application rates at active tillering, jointing, and at anthesis on the grain yield and protein content of 'Minaminokaori' in the field study in southwestern Japan. From the results, we expect to determine the traits on which to base the N application rate at anthesis so as to obtain a sufficiently high protein content of grain for bread making.

## Materials and Methods

The study was conducted during the 2004–2005 crop season on a Gray Lowland 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. Rainfall and mean temperature did not differ greatly from the normal range, with the following exceptions. Rainfall during

Table 1. Dates and N application rates at each growth stage.

| | Seeding | Active tillering | Jointing | Heading | Anthesis | Maturity |
|---|---|---|---|---|---|---|
| Date | 17 November | 28 January | 1 March | 9 April | 18 April | 25 May |
| N application rate (g N $m^{-2}$) | 5 | 0 | 0 | | 0, 2, 4, or 6 | |
| | | or | | | | |
| | | 4 | 2 | | 0, 2, 4, or 6 | |

Table 2. Effects of seeding rate and N application rate on grain yield, its components, and protein content of grain.

| | Grain yield (g $m^{-2}$) | Spikes ($m^{-2}$) | Grains ($spike^{-1}$) | 1000-grain weight (g) | Grain protein content (%) | Days to maturity (days) | Culm length (cm) |
|---|---|---|---|---|---|---|---|
| Seeding rate (seed $m^{-2}$) (A) | | | | | | | |
| 50 | 385 a | 232 b | 42.5 a | 38.7 a | 11.2 a | 189.3 b | 71.8 a |
| 150 | 375 a | 309 a | 31.7 b | 37.6 a | 11.1 a | 189.7 a | 74.7 a |
| N application rate at active tillering and jointing (B) | | | | | | | |
| 0–0N | 278 b | 221 b | 34.2 b | 37.9 a | 10.8 b | 189.0 b | 71.1 b |
| 4–2N | 482 a | 320 a | 40.0 a | 38.3 a | 11.5 a | 190.0 a | 75.4 a |
| N application rate at anthesis (g N $m^{-2}$) (C) | | | | | | | |
| 0 | 366 a | 274 a | 36.4 a | 36.8 c | 9.4 d | 189.2 c | 73.7 a |
| 2 | 379 a | 275 a | 36.8 a | 38.1 b | 10.6 c | 189.3 b | 73.7 a |
| 4 | 389 a | 268 a | 38.0 a | 38.4 ab | 11.7 b | 189.8 a | 73.1 a |
| 6 | 385 a | 264 a | 37.3 a | 39.1 a | 12.9 a | 189.8 a | 72.4 a |
| ANOVA | | | | | | | |
| Seeding rate (A) | NS | * | * | NS | NS | ** | NS |
| N application rate at active tillering and jointing (B) | ** | ** | ** | NS | * | ** | * |
| N application rate at anthesis (C) | NS | NS | NS | * | ** | ** | NS |
| A×B | NS | NS | NS | NS | NS | ** | NS |
| A×C | NS | NS | NS | NS | NS | NS | NS |
| B×C | NS | NS | NS | NS | NS | ** | NS |
| A×B×C | NS | NS | NS | NS | NS | NS | NS |

Values are means of sub-plots.
Means within a column within a treatment by the same letters do not differ significantly ($P<0.05$, LSD).
**, *: significant at $P<0.01$ and $P<0.05$, respectively.

early December 2004 and early May 2005 was 63 and 71 mm higher than normal, whereas that during late April and mid-May 2005 was 54 and 62 mm lower than normal (Fig. 1A). The mean temperatures during early and mid-December 2004 and early April 2005 were 2.4, 3.8, and 2.3ºC higher than normal, whereas that during late February 2005 was 2.3ºC lower than normal (Fig. 1B). The previous crop grown in the field was wheat. We used 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 design with three replicates (Table 1). 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 was broadcasted with 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 with 'Minaminokaori' seed on 17 November 2004 at a rate of 50 or 150 seeds $m^{-2}$. Plants received 0 or 4 g N $m^{-2}$ in the form of ammonium sulfate at active tillering (5.0 leaves), 0 or 2 g N $m^{-2}$ at jointing (7.5 leaves), and 0, 2, 4, or 6 g N $m^{-2}$ at anthesis. 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 (each containing four rows spaced 0.3 m apart).

At anthesis, plants in pre-marked 1.4×1.0 m sample areas were harvested by uprooting. The number of spikes in each sample area was counted. The chlorophyll content index (the "SPAD" value) of the flag leaf of each sample was measured with a chlorophyll meter (SPAD 502, Minolta Co. Ltd., Osaka, Japan). Sampled plants were divided into leaf blades, leaf sheaths plus stems, and spikes. After measurement of the leaf blades with a leaf area meter (LI-3050A/4, LI-COR Ltd., Lincoln, Nebraska, USA), each part was dried at 80ºC for 2 days in a ventilated oven and then weighed to determine its dry weight.

Just before harvesting, culm length was measured, and lodging scores, which ranged from 0 (no lodging) to 5 (serious lodging), were recorded. At maturity, plants from pre-marked 1.4×3.0 m sample areas were harvested by uprooting and were air-dried to a constant weight. The number of spikes in each sample was counted. Dried plants were then 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 the Kjeldahl method (N × 5.70). Protein content of grain was corrected to a 13.5% moisture basis. The number of days to heading was recorded as the number of days from seeding to the stage at which 50% of the heads had completely emerged from the leaf sheath. The number of days to maturity was recorded as the number of days from seeding to the stage when 50% of the spikes were mature. When the *F*-test of the analysis of variance exceeded the 0.05 level of probability, treatment effects were compared using LSD.

## Results

### 1. Grain yield and its components

Grain yield did not differ significantly (P>0.05) between seeding rates (Table 2). The number of spikes per square meter was significantly (P<0.05) lower at a seeding rate of 50 than 150 seeds m$^{-2}$. The number of grains per spike was significantly (P<0.05) higher at 50 than at 150 seeds m$^{-2}$. One-thousand-grain weight did not differ significantly (P>0.05) between seeding rates. Grain yield was significantly (P<0.05) 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). The number of spikes per square meter and that of grains per spike were significantly (P<0.05) higher in 4–2N than in 0–0N. One-thousand-grain weight was not significantly (P>0.05) influenced by nitrogen (N) application rate at active tillering and jointing. Grain yield, the number of spikes per square meter, and that of grains per spike were not significantly (P>0.05) influenced by N application rate at anthesis. One-thousand-grain weight was significantly (P<0.05) higher when 6 g N m$^{-2}$ was applied at anthesis than when 2 or 0 g N m$^{-2}$ was applied.

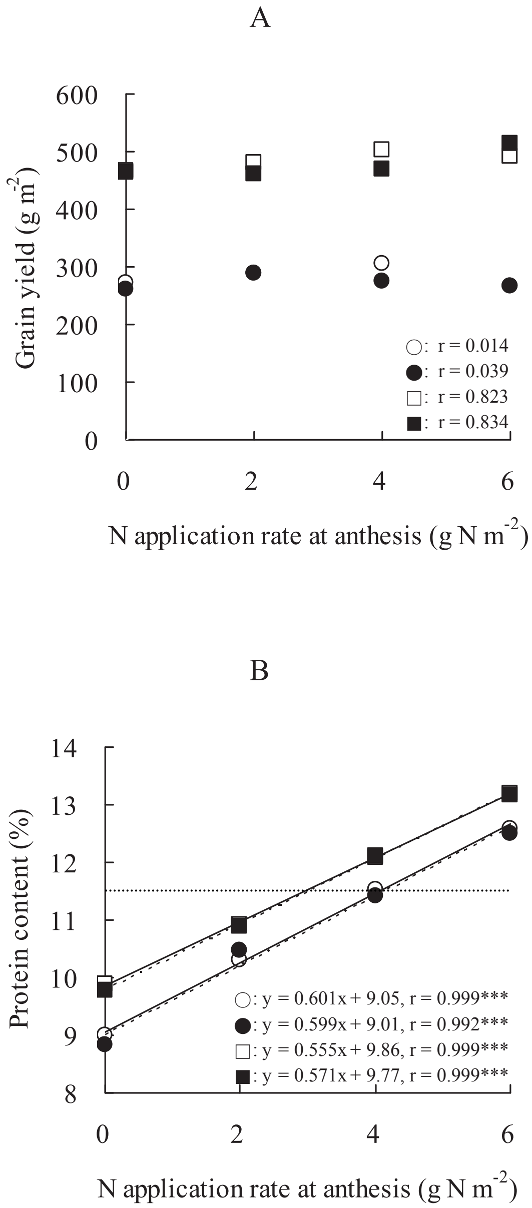


Fig. 2. Correlation of N application rate at anthesis and grain yield (A) and protein content (B).
Seeding rate and N application rate at active tillering and jointing were ○, 50 seeds m$^{-2}$ and 0–0N, respectively; ●, 150 seeds m$^{-2}$ and 0–0N, respectively; □, 50 seeds m$^{-2}$ and 4–2N, respectively; ■, 150 seeds m$^{-2}$ and 4–2N, respectively. ***: significant at *P*<0.001.
The horizontal line in (B) represents 11.5% protein content.

### 2. Protein content of grain

Protein content of grain did not differ significantly (P>0.05) between seeding rates (Table 2). It was

Table 3. Effects of seeding rate and N application rate at active tillering and jointing on growth-related traits at anthesis and days to heading.

| | Spikes ($m^{-2}$) | LAI ($m^2\ m^{-2}$) | SPAD value | Dry weight ($g\ m^{-2}$) | Days to heading (days) |
|---|---|---|---|---|---|
| Seeding rate (seed $m^{-2}$) (A) | | | | | |
| 50 | 248 b | 2.18 a | 40.6 a | 646 a | 143 a |
| 150 | 331 a | 2.19 a | 37.2 b | 744 a | 143 a |
| N application rate at active tillering and jointing (B) | | | | | |
| 0–0N | 247 b | 1.60 b | 35.3 b | 650 b | 143 a |
| 4–2N | 331 a | 2.77 a | 42.5 a | 739 a | 143 a |
| ANOVA | | | | | |
| Seeding rate (A) | | NS | * | NS | NS |
| N application rate at active tillering and jointing (B) | ** | ** | ** | ** | NS |
| A×B | NS | * | NS | NS | NS |

VValues are means of sub-plots.
Means within a column within a treatment by the same letters do not differ significantly ($P<0.05$, LSD).
**, *: significant at $P<0.01$ and $P<0.05$, respectively.

significantly ($P<0.05$) higher in 4–2N than in 0–0N. It was significantly ($P<0.05$) higher when 6 g N $m^{-2}$ was applied at anthesis than when 4, 2, or 0 g N $m^{-2}$ was applied.

### 3. Relationship between grain yield and N application rate at anthesis and between protein content and N application rate at anthesis

Grain yield was not significantly ($P>0.05$) correlated with N application rate in any combination of seeding rate (50 or 150 seeds $m^{-2}$) and N application rate at active tillering and jointing (0–0N or 4–2N) (Fig. 2A). In contrast, protein content of grain was significantly ($P<0.001$) and positively correlated with N application rate at anthesis in all four combinations of seeding rate and N application rate at active tillering and jointing (Fig. 2B). The regression coefficient was generally slightly higher in 0–0N than in 4–2N.

### 4. Days to maturity, culm length, and lodging

The number of days to maturity was significantly ($P<0.05$) higher at 50 than at 150 seeds $m^{-2}$ (Table 2). It was significantly ($P<0.05$) higher in 4–2N than in 0–0N. It was significantly higher when 6 or 4 g N $m^{-2}$ was applied at anthesis than when 2 or 0 g N $m^{-2}$ was applied. Culm length did not differ significantly ($P>0.05$) between seeding rates. It was longer in 4–2N than in 0–0N. It was not significantly ($P>0.05$) influenced by N application rate at anthesis. Lodging was not observed in any treatments (data not shown).

### 5. Growth-related traits at anthesis and days to heading

The number of spikes per square meter was significantly ($P<0.05$) lower at 50 than at 150 seeds $m^{-2}$ (Table 3). It was significantly ($P<0.05$) higher in 4–2N than in 0–0N. Leaf area index (LAI) did not differ significantly ($P>0.05$) between seeding rates. It was significantly ($P<0.05$) higher in 4–2N than in 0–0N. There was a significant ($P<0.05$) interaction between seeding rate and N application rate at active tillering and jointing for LAI. The increase in LAI with the increase in N application rate at active tillering and jointing was smaller at 50 than at 150 seeds $m^{-2}$. The SPAD value was significantly ($P<0.05$) higher at 50 than at 150 seeds $m^{-2}$. It was significantly ($P<0.05$) higher in 4–2N than in 0–0N. Dry weight did not differ significantly ($P>0.05$) between seeding rates. It was higher in 4–2N than in 0–0N. The number of days to heading was not significantly ($P>0.05$) influenced by either seeding rate or N application rate at active tillering and jointing.

## Discussion

We examined the effects of seeding rate, nitrogen (N) application rates at active tillering and jointing, and at anthesis on the grain yield of the bread wheat cultivar, 'Minaminokaori', in southwestern Japan. There are many reports on the effect of seeding rate on grain yield of wheat. In the present study, grain yield was similar at a seeding rate of 50 and 150 seeds $m^{-2}$ (Table 2). Among the grain yield components, the number of spikes per square meter was lower at 50 than at 150 seeds $m^{-2}$. However, the number of grains per spike was higher at 50 than at 150 seeds $m^{-2}$. Iwabuchi et al. (2000) and Fukushima et al. (2004) also reported that grain yield of an early-seeded wheat, 'Iwainodaichi', did not increase with increasing seeding rate in southwestern Japan. Fukushima et al. (2003) found a significant positive correlation between grain yield and leaf area index (LAI) at anthesis in southwestern Japan. In the present study, LAI at anthesis was similar at 50 and 150 seeds $m^{-2}$ (Table 3).

Recently, we reported that N application at active tillering is more effective than that at anthesis in increasing the grain yield of 'Minaminokaori' in southwestern Japan (Nakano et al., 2008). N application at active tillering generally increases the number of spikes per square meter of wheat. In the present study, 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). Among the grain yield components, the number of spikes per square meter and that of grains per spike were higher in 4–2N than in 0–0N. The growth related traits (i.e., LAI, chlorophyll content, and dry weight) at anthesis were higher in 4–2N than in 0–0N (Table 3). However, grain yield was not influenced by N application rate at anthesis (Table 2, Fig. 2A). Thus, N application at active tillering and jointing is more effective than N application at anthesis in increasing the grain yield of 'Minaminokaori'.

High protein content of grain is essential for bread wheat because bread quality is positively correlated with protein content of grain (Gooding et al., 1991). We examined the effects of seeding rate, N application rates at active tillering and jointing, and at anthesis on protein content of grain. Bavec et al. (2002) and Geleta et al. (2002) reported that protein content of grain decreased with increasing seeding rate. In contrast, Campbell et al. (1991) and McLeod et al. (1996) reported that protein content of grain was not influenced by seeding rate. In the present study, grain protein content was similar at 50 and 150 seeds $m^{-2}$ (Table 2). Although the number of grains per spike was higher at 50 than at 150 seed $m^{-2}$, the chlorophyll content at anthesis was also higher at 50 than at 150 seed $m^{-2}$ (Tables 2, 3). Therefore, the balance between chlorophyll content at anthesis and grains per panicle may be important for determining protein content of gain.

Recently, we reported that N application at anthesis is more effective than that at active tillering in increasing the protein content of grain of 'Minaminokaori' in southwestern Japan (Nakano et al., 2008). Protein content of grain was higher in 4–2N than in 0–0N (Table 2). Although the number of grains per spike was higher in 4–2N than in 0–0N, the chlorophyll content at anthesis was much higher in 4–2N than in 0–0N (Tables 2, 3). Protein content of grain was the highest when 6 g N $m^{-2}$ was applied at anthesis, followed by 4, 2, and 0 g N $m^{-2}$ (Table 2, Fig. 2B). The number of grains per spike was not influenced by N application rate at anthesis. Thus, N application at anthesis was more effective than N application at anthesis and jointing in increasing the protein content of grain of 'Minaminokaori'.

Farmers are under pressure to reduce their production costs. Although N application at anthesis markedly increased the protein content of grain, it did not increase grain yield (Table 2). Therefore, farmers should apply the minimum amount of N at anthesis to obtain wheat grains with a sufficiently high protein content (e.g., between 11.5% and 14.0%) for bread making. However, information on the interactions among seeding rate, N application rates at active tillering and jointing, and at anthesis, and their effects on protein content of grain is limited. We found no interaction between seeding rate and N application rate at anthesis in protein content of grain. The SPAD value at anthesis was higher at 50 than at 150 seeds $m^{-2}$, but LAI at anthesis was similar at 50 and 150 seeds $m^{-2}$ and grain protein content was similar at 50 and 150 seeds $m^{-2}$ irrespective of N application rate at anthesis (Fig. 2B). Therefore, sparse seeding of 'Minaminokaori' is recommended. Farmers need not worry about uneven seeding rates when they apply N to 'Minaminokaori' at anthesis. There was also no interaction between N application rate at active tillering and jointing, and that at anthesis on grain protein content (Table 2). LAI and the SPAD value were higher in 4–2N than in 0–0N and protein content of grain was higher in 4–2N than in 0–0N irrespective of N application rate at anthesis. Regardless of seeding rate, 3 g N $m^{-2}$ should be applied at anthesis to obtain a 11.5% protein content of grain if 4 and 2 g N $m^{-2}$ are applied at active tillering and jointing; 4 g N $m^{-2}$ should be applied at anthesis if 0 and 0 g N $m^{-2}$ are applied at active tillering and jointing, respectively (Fig. 2B). This result, combined with the result of Nakano et al. (2008), indicate that 3 g N $m^{-2}$ or more should be applied at anthesis when 'Minaminokaori' grown with standard N application rates (5 g N $m^{-2}$ at seeding, 4 g N $m^{-2}$ at active tillering, 2 g N $m^{-2}$ at jointing). Wheat plants translocate nitrogen from leaf and stem at anthesis to the grains during the ripening period. The amount of nitrogen in leaf and stem at anthesis is related to LAI and the SPAD value. Therefore, both LAI and the SPAD value may be important traits for determining the optimal N application rate at anthesis. Farmers should apply N according to LAI and the SPAD value at anthesis. However, further study is needed to define the optimal amount of N application at anthesis according to LAI and the SPAD value at anthesis.

Eguchi et al. (1969) reported that topdressing sometimes delays maturity and increases lodging, thus decreasing grain quality. In the present study, the difference in the number of days to maturity was no more than 2 days (data not shown). Furthermore, higher N application rates at anthesis did not increase lodging, possibly because culm length did not increase. Takayama et al. (2004) and Nakano et al. (2008) reported similar results in western and southwestern Japan, respectively. Thus, N application at anthesis does not appear to delay maturity or increase lodging.

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