

# Differences Between Rice and Wheat in Temperature Responses of Photosynthesis and Plant Growth

Takeshi Nagai and Amane Makino\*

Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai, 981-8555 Japan

The temperature responses of photosynthesis (A) and growth were examined in rice and wheat grown hydroponically under day/night temperature regimes of 13/10, 19/16, 25/19, 30/24 and 37/31°C. Irrespective of growth temperature, the maximal rates of A were found to be at 30–35°C in rice and at 25–30°C in wheat. Below 25°C the rates were higher in wheat, while above 30°C they were higher in rice. However, in both species, A measured at the growth temperature remained almost constant irrespective of temperature. Biomass production and relative growth rate (RGR) were greatest in rice grown at 30/24°C and in wheat grown at 25/19°C. Although there was no difference between the species in the optimal temperature of the leaf area ratios (LARs), the net assimilation rate (NAR) in rice decreased at low temperature (19/16°C) while the NAR in wheat decreased at high temperature (37/31°C). For both species, the N-use efficiency (NUE) for growth rate (GR), estimated by dividing the NAR by leaf-N content, correlated with GR and with biomass production. Similarly, when NUE for A at growth temperature was estimated, the temperature response of NUE for A was similar to that of NUE for GR in both species. The results suggest that the difference between rice and wheat in the temperature response of biomass production depends on the difference in temperature dependence of NUE for A.

**Keywords:** Biomass production • N-use efficiency • *Oryza sativa* L • Photosynthesis • Temperature • *Triticum aestivum* L.

**Abbreviations:** A, photosynthetic assimilation rate; GR, growth rate; LAR, leaf area ratio; LWR, leaf weight ratio; NAR, net assimilation rate; NUE, N-use efficiency; PPFD, photosynthetic photon flux density; RGR, relative

growth rate; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; SLA, specific leaf area.

## Introduction

Rice and wheat are the two most commercially important crops, providing food for >90% of the world's population. They were domesticated in different climates and so differ significantly in their growth conditions: rice is a tropical crop while wheat is a winter crop. As a result, cool summers in Japan often lead to decreased yields of rice, and several studies attempted to elucidate the mechanism whereby low temperature damages photosynthesis and growth (Kabaki et al. 1982, Maruyama et al. 1990, Ohashi et al. 2000, Hirotsu et al. 2004, Hirotsu et al. 2005, Suzuki et al. 2008). Nevertheless, growth responses to temperature are not well known in either rice or wheat, even though these species have been used as model plants for many years. Indeed, their optimal growth temperatures remain uncertain, and the factors that determine the differences in temperature-related growth traits between rice and wheat are unclear. This research is increasingly urgent in an era of global climate change, when most cereal yields are predicted to decrease as a result of an increase in temperature (Peng et al. 2004, Lobell and Field 2007).

Changes in growth temperature induce morphological responses and alternations in biomass allocation (Boese and Huner 1990, Equiza et al. 2002, Atkin et al. 2006a). Such changes result in inherent differences between plant species in the temperature dependence of the relative growth rate (RGR) (Loveys et al. 2002, Storkey 2004). There are some studies attempting to explain the temperature response of RGR as a function of underlying growth indices such as net

\*Corresponding author: E-mail, makino@biochem.tohoku.ac.jp; Fax, +81-22-717-8765.

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assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR). Poorter and co-workers (Poorter and Remkes 1990, Poorter et al. 1990) studied 24 wild species differing in RGR and found that fast-growing species allocate more carbon to their leaves and show higher LAR. In contrast, Loveys et al. (2002) observed that variation in NAR played a greater role in determining interspecific differences in RGR between temperature treatments. Thus, the effects of growth temperature on RGR and its underlying growth indices appear to differ amongst species, and the mechanisms of growth temperature response still remain unclear.

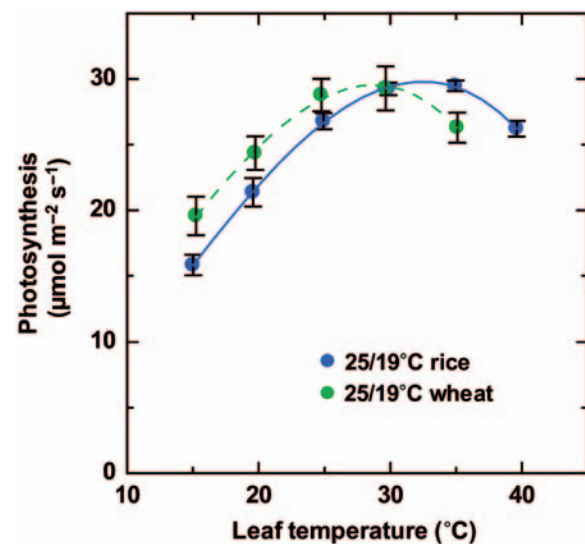
Photosynthetic capacity is also strongly influenced by temperature (for a recent review, see Sage and Kubien 2007). Although rice and wheat are both  $C_3$  plants, the photosynthetic assimilation rate ( $A$ ) at 25°C is higher in wheat than rice, for the same N content. This is mainly because wheat has a greater specific activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Makino et al. 1988) and a higher content of Cyt  $f$  (Sudo et al. 2003). However, differences between the two species have not previously been examined in respect of the response of  $A$  to temperature change, the effects of growth temperature on  $A$ , and N-use efficiency (NUE) for  $A$ .

In the present study, we aimed to characterize the differences between rice and wheat in both the photosynthetic and growth responses to temperature change. We grew the plants hydroponically under five different day/night temperature regimes: 13/10 (wheat only), 19/16, 25/19, 30/24 and 37/31°C. Initially, temperature responses of  $A$  were examined in fully expanded, young leaves from each growth regime. Secondly, we investigated dry mass production and biomass allocation and carried out growth analyses. We addressed the following questions. (i) What are the differences between rice and wheat in their temperature responses of  $A$  and growth? (ii) Are there any relationships between the temperature responses of individual leaves and whole plants?

## Results

### Differences between rice and wheat in temperature responses of photosynthesis

**Fig. 1** shows the temperature responses of  $A$  at  $pCi = 28$  Pa in rice and wheat grown at 25/19°C. The maximal  $A$  was found to be at 30–35°C in rice and 25–30°C in wheat. Below 25°C the rates of  $A$  were higher in wheat, while above 30°C they were higher in rice. Next, we examined the effects of initial growth temperature regime on the temperature responses of  $A$  (**Fig. 2**). In rice,  $A$  per unit leaf area tended to be greatest in the plants grown at the lowest temperature, 19/16°C. Similarly, in wheat,  $A$  was greatest in the plants grown at 13/10°C. However, when the responses of  $A$  were normalized to the maximal  $A$ , the photosynthetic responses to temperature



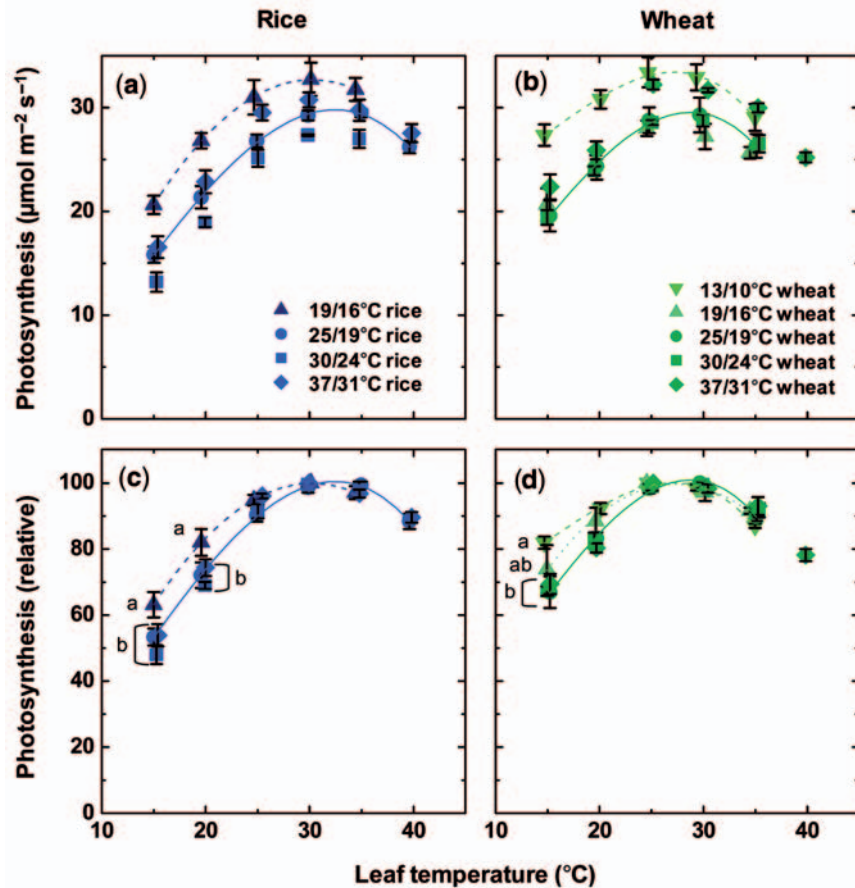
**Fig. 1** Temperature response of  $A$  at  $pCi = 28$  Pa in leaves of rice (blue circle) and wheat (green circle) grown at a day/night temperature regime of 25/19°C. Measurements were made at a PPFD of 1,500  $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ . The data were fitted by a third-order polynomial.

were similar for each growth regime, except in rice grown at 19/16°C and wheat grown at 13/10°C, which showed significantly higher  $A$  at 15–20°C (**Fig. 2c, d**). The wheat grown at 19/16°C exhibited  $A$  intermediate between the 13/10°C treatment and the others (**Fig. 2d**,  $P < 0.05$ ). Thus, the optimal temperature of  $A$  was not altered by growth temperature, but the plants grown under lower temperatures had relatively higher  $A$  at low temperatures. This phenomenon was observed for both species.

**Fig. 3** shows  $A$  measured at each daytime growth temperature. Surprisingly,  $A$  per unit leaf area remained almost constant across the wide range of temperature, irrespective of treatments and species.

### Leaf properties

Total leaf-N contents were 30–40% greater in the rice grown at 19/16°C and in the wheat grown at 13/10°C (**Fig. 4a, b**, respectively,  $P < 0.05$ ), which also exhibited higher  $A$  (see **Fig. 2a, b**). Similarly, both species grown at the highest temperature regime, 37/31°C, also had greater total leaf-N content, and tended to show slightly higher  $A$ . These increases in total leaf-N contents per unit leaf area in the plants grown at high temperature as well as those grown at low temperature led to no differences in the  $A$  at growth temperature between treatments across the wide range of temperature (**Fig. 3**). This may be one compensation mechanism for decreased photosynthesis at low and high temperatures. Indeed, these characteristics always led to an increase in Rubisco content (**Fig. 4c, d**). Similar responses were found for Chl content in both species, except in the wheat grown at 37/31°C (**Fig. 4g, h**).



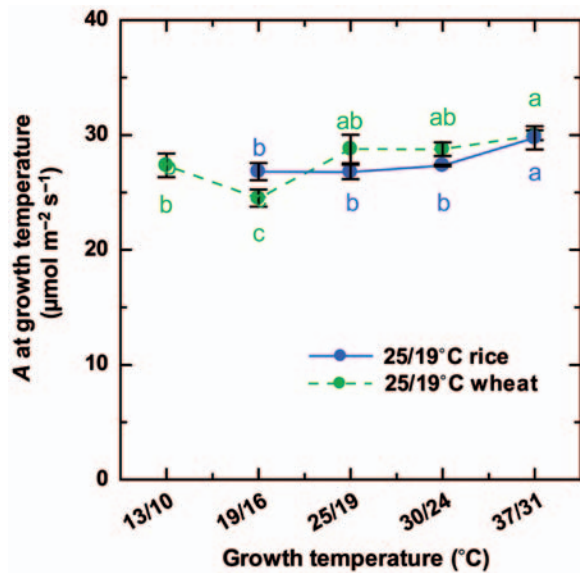
**Fig. 2** Temperature responses of A (a, b) and relative A (c, d) at  $pC_i = 28$  Pa in leaves of rice (blue symbols) and wheat (green symbols) grown at day/night regimes of 13/10°C (inverted triangle), 19/16°C (triangle), 25/19°C (circle), 30/24°C (square) and 37/31°C (diamond). Relative A is normalized to the maximum A for each treatment as 100. Measurements were made at a PPFD of  $1,500 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$ . Data represent means  $\pm$  SD ( $n = 3-5$ ). No bar indicates the SD was within the size of the symbols. The data were fitted by a third-order polynomial. Different letters indicate statistical difference (where a is greater than b) at  $P < 0.05$  between temperature treatments (Tukey–Kramer’s HSD test).

However, the degree of the Chl responses was smaller than those of Rubisco. The ratio of Rubisco to total leaf-N content tended to increase in both species with increasing total leaf-N, and this ratio was also higher in the plants grown at low and high temperature (Fig. 4e, f). However, the ratio of Chl to total leaf-N in rice increased with increasing temperature, and in wheat the ratio was lower in the plants grown at low and high temperature (Fig. 4i, j). Since the Chl *a/b* ratio decreased with increasing temperature in rice, this indicated that Chl *b* content was greater in plants grown at high temperature (Fig. 4g). On the other hand, Chl *b* content in wheat was greater in the plants grown at medium temperature (Fig. 4h).

### Biomass production and N content at the whole plant level

Wheat and rice have inherently different growth rates, making interspecific comparisons of growth rate problematic.

Therefore, according to the results of the preliminary experiments, we set the sampling day for each species in order to obtain an equal biomass at final harvest when grown at 25/19°C (day 63 for rice and day 35 for wheat, see Fig. 5a and b). The maximal biomass production was found to be at 30/24°C in rice and 25/19°C in wheat (Fig. 5). In rice, biomass decreased dramatically in the plants grown at 19/16°C, which had 16% the mass of the plants grown at 25/16°C. Rice could not grow at 13/10°C (data not shown). In wheat, a large decrease in biomass was found for the plants grown at 37/31°C which had 13% the mass of the plants grown at 25/16°C. The total N content of the plant showed a similar pattern to biomass production in both species. However, N content on a biomass basis was greater in the plants grown at low and high temperature. This mainly led to an increase in total leaf-N content per unit leaf area in these treatment regimes in both species (see Fig. 4a, b).

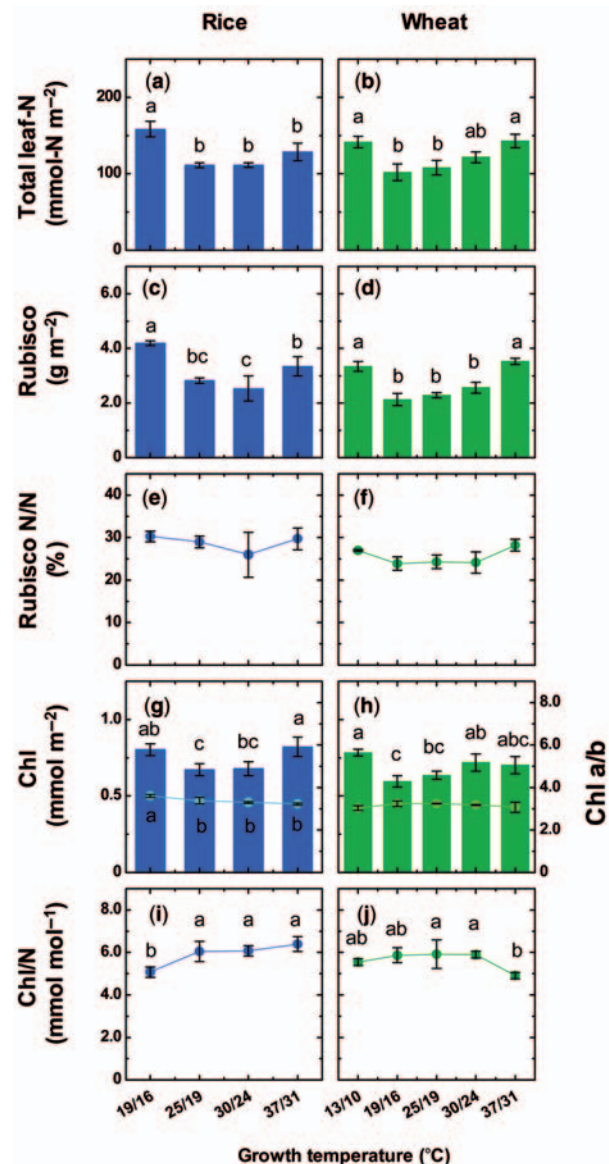


**Fig. 3** A measured at each daytime growth temperature in leaves of rice (blue symbols) and wheat (green symbols) grown at the indicated temperature. Measurements were made at a PPFD of  $1,500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  and a  $p\text{Ci}$  of 28 Pa. Data represent means  $\pm$  SD ( $n = 3-5$ ). No bar indicates the SD was within the size of the symbols. Different letters indicate statistical difference (where a is greater than b) at  $P < 0.05$  between temperature treatments (Tukey–Kramer's HSD test).

### Plant growth analysis

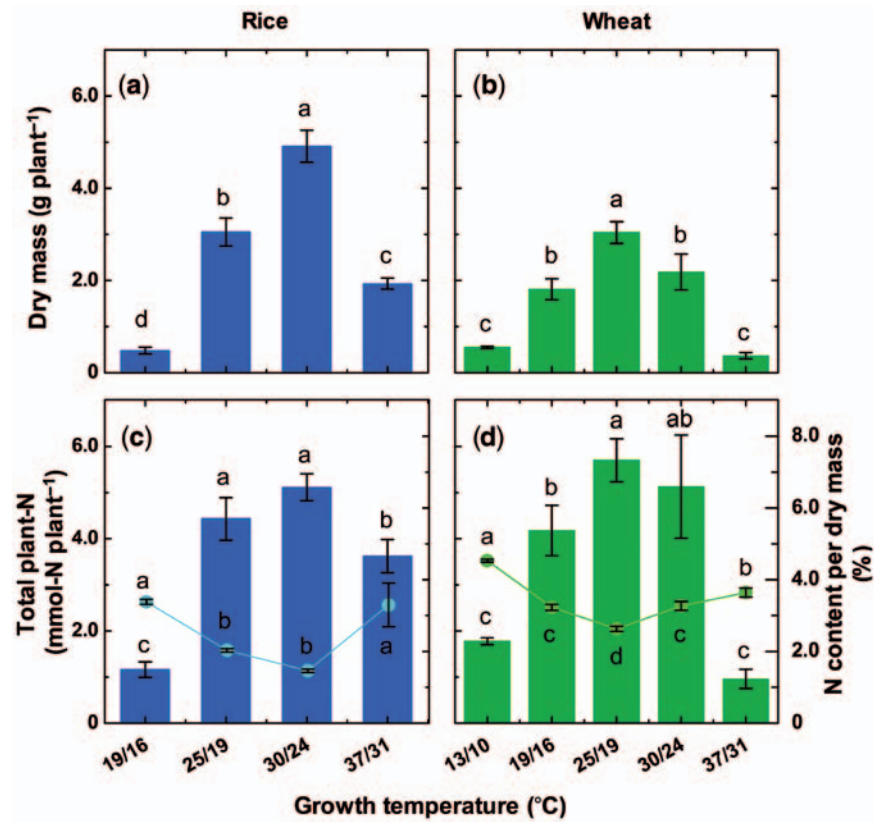
**Fig. 6** shows the results of growth analyses in rice between 21 and 42 d and between 42 and 63 d, and in wheat between 7 and 21 d and between 21 and 35 d. Irrespective of growth temperature, RGR was generally higher in wheat than in rice, confirming wheat's more rapid growth rate (**Fig. 6a–d**). Although NAR was higher in rice than in wheat (**Fig. 6e–h**), LAR was consistently higher in wheat (**Fig. 6i–l**), as a result of higher values for both SLA and LWR (**Fig. 6m–t**). These results indicate that in comparison with rice, wheat both has thinner leaves and allocates more biomass to its leaves, allowing it to realize a more rapid growth rate.

In both rice and wheat, the temperature responses of RGR in the first half of the experiment (21–42 and 7–21 d in rice and wheat, respectively, **Fig. 6a, c**) tended to be more similar to the temperature response of biomass at final harvest (**Fig. 5**). These results indicated that the growth rate at early stages was a relatively important determinant for total biomass production. LAR was maximal at 30/24°C in both species, except for wheat in the first half of the growth period which was slightly higher at 37/31°C than at 30/24°C. The temperature responses of SLA tended to mimic those of LAR, showing a decline at 37/31°C except for wheat in the first half of the growth period. LWR always increased with increasing growth temperature in both species (**Fig. 6q–t**).



**Fig. 4** Effects of growth temperature on total leaf N content (a, b), Rubisco content (c, d), the ratio of Rubisco-N to leaf-N (e, f), Chl content and Chl *a/b* ratio (g, h) and the ratio of Chl to leaf-N content (i, j) in rice (blue column) and wheat (green column) grown at the indicated day/night temperature. Data represent means  $\pm$  SD ( $n = 3-5$ ). Different letters indicate statistical difference (where a is greater than b) at  $P < 0.05$  between temperature treatments (Tukey–Kramer's HSD test). No letter indicates no significant difference (e, f and Chl *a/b* in h).

Thus, no differences are noted between rice and wheat with regard to the temperature response of leaf expansion. The increase in LAR in the rice grown at 30/24°C led to an increase in RGR, while the increase in LAR in the wheat grown at 30/24°C did not, because decreases in NAR at high temperatures exceeded the effects of increases in LAR (**Fig. 6c, d, g, h, k, l**).



**Fig. 5** Effects of growth temperature on biomass production (a, b) and total plant-N and N content per dry weight (c, d) in rice (blue column and symbols) and wheat (green column and symbols) at day 63 and day 35 after germination, respectively. Data represent means  $\pm$  SD ( $n = 4$ ). Different letters indicate statistical difference (where a is greater than b) at  $P < 0.05$  between temperature treatments (Tukey–Kramer's HSD test).

In both species, declines in RGR were always associated with decreases in NAR. NAR decreased to a great extent at low temperature (19/16°C) in rice and at high temperature (37/31°C) in wheat, and led to a large decline in biomass (Fig. 4). When the NUE for growth rate (GR) was calculated by dividing the NAR by each N content in the leaf blade (see Equation 6 in Materials and Methods, Makino et al. 2000), it showed similar correlations with final biomass production in both species (Fig. 6x, y).

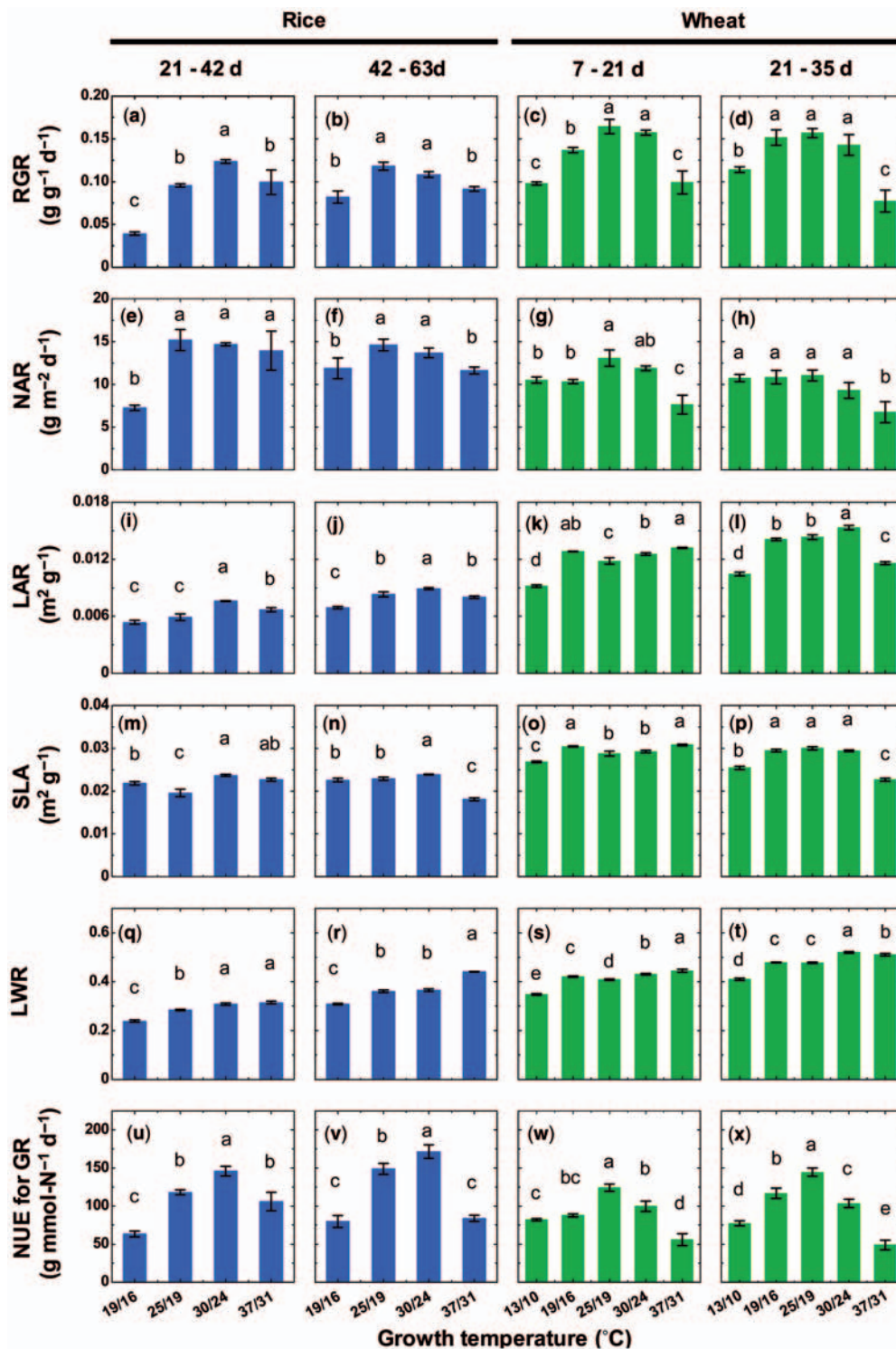
The relationships between NAR, LAR, NUE for GR and RGR were analyzed (Fig. 7). Although all growth indices were positively correlated with RGR, the correlation coefficients of NAR and NUE for GR were appreciably higher than that of LAR. However, NUE was also higher in rice than in wheat for the same RGR.

### Relationships between photosynthesis and whole plant growth

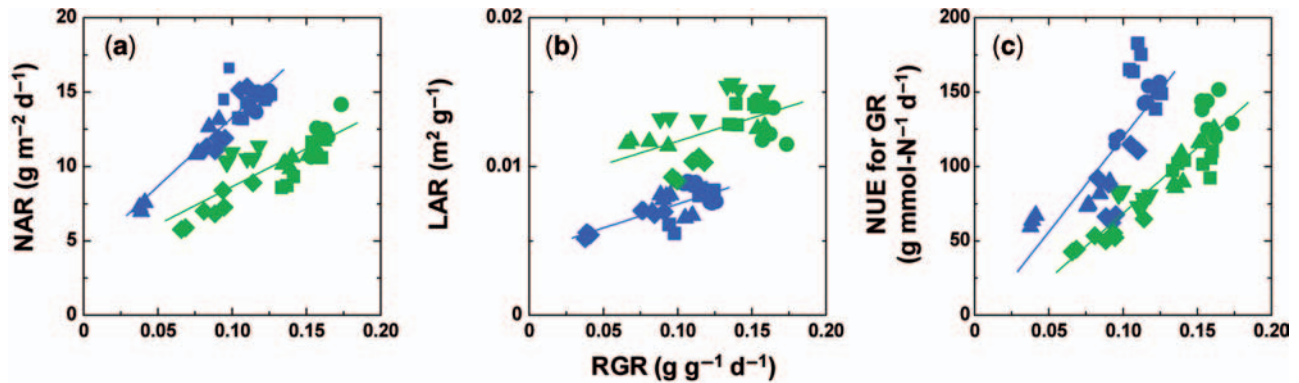
To examine the relationship between A and biomass production, we compared A at each daytime growth

temperature (Fig. 3) with NAR (Fig. 6f, h). Whereas A at the growth temperature in both species remained almost constant irrespective of the treatment regimes, the temperature responses of NAR in rice peaked at low and high temperatures, and NAR in wheat declined significantly above 37/31°C. In addition, whereas A at growth temperature did not differ between the two species, NAR was higher in rice than in wheat. Thus, the temperature responses of A at growth temperature were not necessarily consistent with those of NAR in each species.

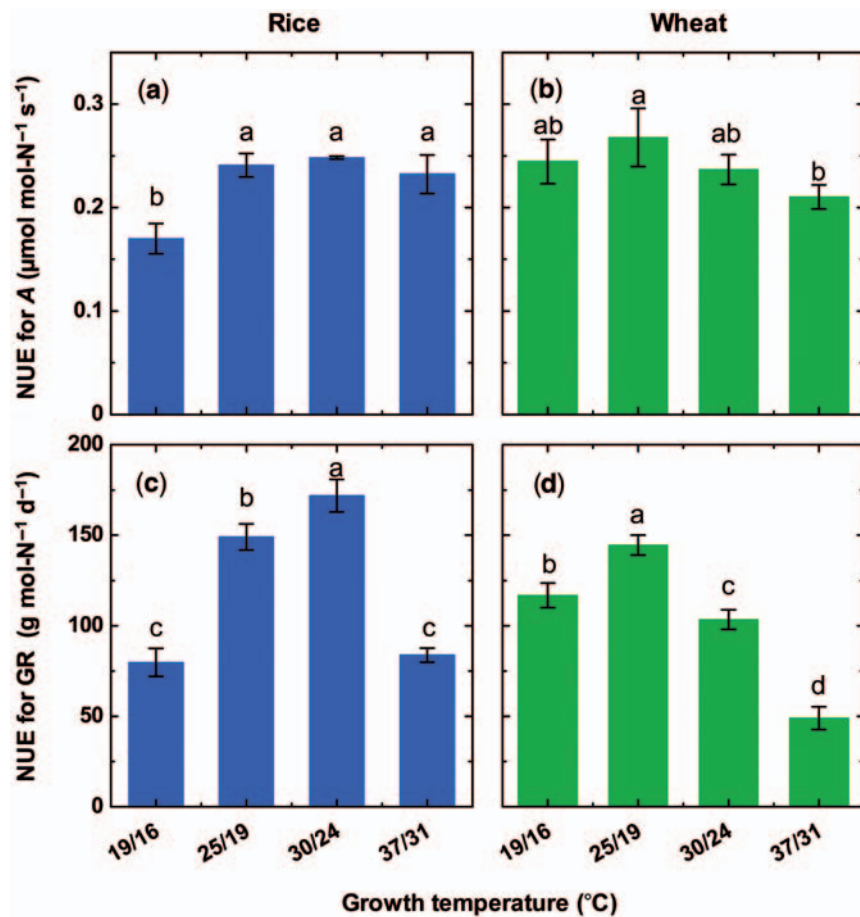
We next compared the temperature responses of NUE for A at the level of a single leaf with those for biomass production rate at the level of the whole plant (NUE for GR). The NUE for A was calculated by dividing A at growth temperature by the N content of each leaf which had been used for the gas exchange measurement (Fig. 8). Overall responses to temperature between NUE for A and NUE for GR were similar in both species. However, the peak in temperature response of NUE for A was flatter than that in NUE for GR in both species.



**Fig. 6** Effects of growth temperature on RGR (a–d), NAR (e–h), LAR (i–l), SLA (m–p), LWR (q–t) and NUE for GR (x, y) in rice (blue column) and wheat (green column) grown at the indicated day/night temperature. Data represent means  $\pm$  SD ( $n = 3-5$ ). Different letters indicate statistical difference (where a is greater than b) at  $P < 0.05$  between treatments within each growth analysis (Tukey–Kramer’s HSD test).



**Fig. 7** Relationships between NAR (a), LAR (b) and NUE for GR (c), and RGR in rice (blue symbols) and wheat (green symbols). Plants were grown at day/night regimes of 13/10°C (inverted triangle), 19/16°C (triangle), 25/19°C (circle), 30/26°C (square) and 37/31°C (diamond). For NAR,  $y = 93x + 4.03$ ,  $r^2 = 0.90$  (rice);  $y = 51x + 3.54$ ,  $r^2 = 0.81$  (wheat). For LAR,  $y = 0.032x + 0.43$ ,  $r^2 = 0.69$  (rice);  $y = 0.031x + 0.86$ ,  $r^2 = 0.52$  (wheat). For NUE for GR,  $y = 1270x - 7.44$ ,  $r^2 = 0.80$  (rice);  $y = 884x - 19.9$ ,  $r^2 = 0.90$  (wheat).



**Fig. 8** Effects of growth temperature on NUE for A and NUE for GR in rice (blue column) and wheat (green column) grown at the indicated temperature. NUE for A was estimated by dividing A at the growth temperature (Fig. 3) by total leaf-N content (Fig. 4). NUE for GR values were taken from Fig. 6v and x. Data represent means  $\pm$  SD ( $n = 3-5$ ). Different letters indicate statistical difference (where a is greater than b) at  $P < 0.05$  between temperature treatments (Tukey-Kramer's HSD test).

## Discussion

### Which factors determine the difference between rice and wheat in the optimal temperature of photosynthesis?

Our previous comparative studies of photosynthesis in rice and wheat showed that  $A$  at 25°C is higher in wheat than in rice (Sudo et al. 2003). In the present study, however, our results clearly showed that the optimal temperature of  $A$  is always higher in rice than in wheat and that the rates <25°C were higher in wheat and rates >30°C were higher in rice (Figs. 1, 2). In agreement with our results, there have been some reports pointing out that plants from cool habitats have relatively higher  $A$  at cool temperatures while plants from warm habitats show higher  $A$  at warm temperatures (for a review, see Berry and Björkman 1980). However, the factors that determine such differences are still largely unknown.

Under current atmospheric CO<sub>2</sub> and O<sub>2</sub> conditions, as long as Pi regeneration is not limiting, Rubisco plays a predominant role in  $A$  in C<sub>3</sub> plants, particularly near the thermal optimal of  $A$  (Hikosaka 1997, Cen and Sage 2005, Hikosaka et al. 2006, Yamori et al. 2006, Ishikawa et al. 2007, Makino and Sage 2007, Suzuki et al. 2007, Yamori et al. 2008). Among these studies, Makino and Sage (2007) found that  $A$  at cool temperatures (15–20°C) is predominantly limited by Rubisco capacity. In addition, Sage (2002) observed higher specific activities ( $K_{\text{cat}}$ ) of Rubisco in C<sub>3</sub> species originating from cool environments compared with those from warm environments. Consistent with this, Makino et al. (1988) previously reported that the  $K_{\text{cat}}$  of Rubisco is about 50% higher in wheat than in rice. Thus, one possible explanation is that the higher Rubisco activity in wheat leads to higher  $A$  at cool temperatures.

At high temperatures, however, the principle factor(s) limiting  $A$  remain unclear. Several researchers have pointed out that Rubisco activase activity limits  $A$  at high temperature because of the inherently high thermal lability of this enzyme (Feller et al. 1988). In fact, Salvucci and Crafts-Brandner (2004) suggest that differences in the temperature response of  $A$  at high temperatures are reflected by the difference in the thermal properties of Rubisco activase. On the other hand, it has been reported that electron transport activity becomes limiting at high temperatures (Yamasaki et al. 2002, June et al. 2004, Cen and Sage 2005). PSII is also sensitive to high temperature (Mawson and Cummins 1989, Yamasaki et al. 2002, Wise et al. 2004). However, at present, there are no available data describing differences in the thermal properties of either activase or electron transport between rice and wheat.

### Photosynthetic acclimation to temperatures

Some previous studies have shown alternations in the thermal optima of  $A$ , depending on growth temperatures

(Berry and Björkman 1980, Boese and Huner 1990, Yamasaki et al. 2002, Yamori et al. 2005, Way and Sage 2008). These alternations may be caused by changes in leaf-N content as well as N use within a leaf depending on growth temperature (Hikosaka 1997). In fact, some changes in N partitioning among photosynthetic components have been observed with changes in the growth temperature (Makino et al. 1994b, Hikosaka 1997, 2005, Yamori et al. 2005). In the present study, however, we found that the optimal temperature was not shifted according to growth treatment regimes in either species (Fig. 2). In addition,  $A$  per unit leaf area measured at each daytime growth temperature remained almost constant irrespective of growth temperature regimes and species (Fig. 3). This phenomenon is considered as a homeostatic response to maintain a certain rate of  $A$  at the growth temperature (for reviews, see Berry and Björkman 1980, Hikosaka et al. 2006), and has been observed in several species (Yamori et al. 2005, Hikosaka et al. 2006, Yamori et al. 2009). For example, leaves grown at low temperature are always associated with increases in leaf-N content (Yamori et al. 2005, also Fig. 4). This may have been a compensation mechanism developed for decreased  $A$  at low temperature. In the present study, the plants grown at the lowest temperatures, i.e. rice grown at 19/16°C and wheat grown at 13/10°C, exhibited a lower suppression of  $A$  at low temperatures (15–20°C) compared with rice grown at its normal temperature ( $P < 0.05$ ) (Fig. 2). Such low-temperature-grown plants had greater Rubisco and N contents than those grown at their normal temperatures (Fig. 4). This increase in Rubisco content may also represent a mechanism of photosynthetic acclimation to cool temperatures because of the predominant limitation of Rubisco at low temperatures (Makino and Sage 2007). Thus, although no alternations in the thermal optima of  $A$  were found in our study, photosynthetic acclimation to low temperatures did occur in both species. Recently, Yamori et al. (2009) reported that such increases in Rubisco content can be observed in several cold-tolerant species with a high degree of photosynthetic homeostasis, while some species with a low degree of homeostasis do not show such changes in Rubisco.

Surprisingly, we also found relative increases in Rubisco and leaf-N contents for both rice and wheat grown at high temperature (Fig. 4). These changes led to a photosynthetic homeostasis to high temperature (Fig. 3). Interestingly, however, the increase in Rubisco content did not lead to an increase in  $A$  at low temperature (Figs. 2, 4). The reason for this is not clear, but one possible explanation is Pi regeneration limitation occurring in plants grown at high temperature. In species adapted or acclimated to cool conditions, Pi regeneration capacity does not always limit  $A$  at low temperature (Sage and Kubien, 2007, Ishikawa et al. 2007). In fact, Makino et al. (1994b) found an increase in the activities of sucrose synthesis limiting Pi regeneration in rice grown at low temperature. Therefore, it is possible that



Pi regeneration limitation occurs in the plants grown at high temperature.

### Differences in growth rate and its optimal temperature between rice and wheat

The growth rate was appreciably faster in wheat than in rice. According to our results, wheat both has thinner leaves and allocates more biomass to leaves, and therefore exhibits a higher LAR compared with rice, allowing it to realize a more rapid growth rate (Fig. 6). A similar relationship has been reported by Poorter and co-workers with comparative studies of 24 wild species differing in RGR (Poorter and Remkes 1990, Poorter et al. 1990). They observed that fast-growing species allocated more carbon to leaves than slow-growing species and that higher LAR in fast-growing species allows plants to fix more carbon per unit plant weight, with a positive correlation between LAR and RGR. Thus, LAR is an important factor in the inherent growth rate. In addition, stimulation of LAR by elevated temperature is likely to be a common phenomenon in other species (cf. the rose, see Ushio et al. 2008), including rice and wheat in the present study (Fig. 6). However, LAR is not a determinant for the difference between rice and wheat in the temperature response of GR.

The optimal temperature for biomass production was higher in rice than in wheat (Fig. 5). The optimal temperature for A was also higher in rice than in wheat (Fig. 1). This is entirely expected, since Japonica type rice is a subtropical species, whilst wheat is adapted to temperate and Mediterranean climates. Although LAR was an important determinant for GR, no difference in the temperature response of LAR was found between the two species (Fig. 6). On the other hand, declines in NAR were always associated with decreases in RGR in both species (Fig. 6) and a more positive correlation was observed between NAR and RGR (Fig. 7). NAR decreased dramatically at low temperature in rice and at high temperature in wheat, and led to a large decline in biomass. These results indicated that differences in the temperature response of GR between rice and wheat are caused more by a difference in the temperature response of NAR. Loveys et al. (2002) and Storkey (2004) also reported that variation in NAR plays an important role in differences in RGR at low temperatures, and the importance of SLA decreased as the temperature decreased. Kurimoto et al. (2004) studied rice and wheat grown at different temperatures, and observed that NAR was mainly responsible for the difference in RGR between the two species. Furthermore, we found that NUE for GR was also highly correlated with RGR and biomass (Figs. 6, 7). These results indicate that although decreased biomass production at low and high temperatures was associated with an increase in N content as a compensation mechanism (see Fig. 4), biomass production at low and high temperatures is strongly determined by the lower NUE.

### N-use efficiency determines biomass production depending on growth temperature

Whereas A at the growth temperature remained almost constant irrespective of temperature, a small dependency on temperature was observed for NUE for A at the growth temperature (Fig. 8). In addition, this response tended to be similar to those of biomass production and RGR. Therefore, we consider that NUE for A determines biomass production depending on the growth temperature in both species. However, the peak in temperature response of NUE for A was flatter than that of NUE for GR. There are some possible reasons for this difference between A and GR. For example, photosynthate-use efficiency for growth may have been low at lower temperatures. A large accumulation of starch and sucrose is observed in several species when grown at lower temperatures, including rice (Park and Tsunoda 1974), spinach (Guy et al. 1992) and *Solanum* species (Chen and Li 1980). Such accumulation of carbohydrates can be an indicator of the inefficiency with which photosynthate is used for growth (for a review, see Stitt and Schulze 1994). Thus, at lower temperatures, A exceeds GR, resulting in an accumulation of starch and sucrose. At high temperatures, respiration increases relative to A (Atkin et al. 2006b, Atkin et al. 2007). The ratio of respiration to A is higher at the level of the whole plant than at the level of an individual leaf. For example, in field-grown rice, Cock and Yoshida (1972) estimated the ratio of respiration to photosynthesis to be about 40%. Okawa et al. (2002) reported that loss of carbon by respiration reached 25–30% of biomass production in rice. Atkin et al. (2007) observed the ratio of respiration to gross A to be 23–38% depending on growth temperature in two *Plantago* species. In contrast, they found that this respiration ratio was much smaller at the level of an individual leaf, typically 5–15% in the same species (Atkin et al. 2006b). In addition, respiration during the day was further suppressed (2–8% to gross A). Similar ratios (5–10%) at the leaf level were observed for spinach (Yamori et al. 2005), sweet potato (Cen and Sage 2005) and rice (Makino and Sage 2007). Thus, such factors may have stimulated a larger dependency on temperature of GR than that of A.

### Conclusion

In the present study, we found that the optimal temperature for both photosynthesis and plant growth is higher in rice than in wheat. The temperature response of NAR exhibited clear differences between the species, while there were no differences in the temperature responses of LAR. In addition, we noted correlations between NAR and RGR in both species. This suggested that the differential temperature response of NAR is the primary determinant of growth rate, but A at the growth temperature at the level of an individual leaf remained almost constant in both species irrespective of temperature. When NUE for A was estimated, however,

the temperature response of NUE for A was similar to that of biomass production and GR. Thus, we conclude that in both species, NUE for A determines biomass production depending on growth temperature.

## Materials and Methods

### Plant material and growth conditions

Rice (*Oryza sativa* L. cv. Notohikari) and wheat (*Triticum aestivum* L. cv. Ias) plants were grown hydroponically with continuous aeration in an environmentally controlled growth chamber (Makino et al. 1994a). The chamber had a 15 h photoperiod, 60% relative humidity and a photosynthetic photon flux density (PPFD) of 1,000  $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$  at plant level during the daytime. Day/night growth temperatures in the chamber were maintained at 13/10 (wheat only), 19/16, 25/19, 30/24 and 37/31°C, for replicate sets of plants grown sequentially. Rice was not grown at 13/10°C. The basal hydroponic solution used for both species was previously described by Makino et al. (1988). Gas exchange measurements were performed on young, fully expanded leaves of 63- to 84-day-old plants and 35- to 49-day-old plants in rice and wheat, respectively. Growth analyses were carried out in rice plants from 21 to 42 d and from 42 to 63 d after germination, and in wheat plants from 7 to 21 d and from 21 to 35 d. Since the growth rate was faster in wheat than in rice, the sampling day was set for each species in order to obtain equal biomass at final harvest between when grown at 25/20°C (day 63 for rice and day 35 for wheat, see Fig. 5).

### Gas exchange measurements

Gas exchange rates were measured with a portable gas exchange system (LI-6400, Li-Cor, Lincoln, NE, USA) according to Hirotsu et al. (2004). The relative humidity in the chamber was maintained at 60–70% with a dew point generator (LI-610, Li-Cor). Irradiance was provided by a cool-light source (PCS-HRX, Nippon PI, Tokyo, Japan) and adjusted to a PPFD of 1,500  $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$  at the leaf surface in the chamber. A was measured sequentially at 25, 30, 35, (40, partly), 20 and 15°C leaf temperature. All photosynthetic measurements were made at a  $p\text{C}_i$  of 28 Pa. Gas exchange parameters were calculated according to the equations of von Caemmerer and Farquhar (1981).

### Determinations of total leaf-N, Chl and Rubisco

After the completion of gas exchange measurements, the leaf blade was homogenized with a chilled pestle and mortar in 50 mM Na-phosphate buffer (pH 7.0) containing 2 mM Na-iodoacetate, 0.8% (v/v) 2-mercaptoethanol and 5% (v/v) glycerol (Makino et al. 1994a). Total leaf-N was determined from an aliquot of the homogenate before centrifugation. The N content was determined with Nessler's reagent after

Kjeldahl digestion (Makino et al. 1994a). The remaining homogenate was used for the determination of Rubisco and Chl contents according to Makino et al. (1994a). For Rubisco determination, a Triton X-100 solution to a final concentration of 0.1% (v/v) was added to an aliquot of leaf homogenate. After centrifuging the homogenate at 15,000 $\times g$  for 5 min at 4°C, lithium dodecylsulfate [1.0% (v/v)] was added, and the sample boiled at 100°C for 1 min, then analyzed by SDS-PAGE. The Rubisco content was determined spectrophotometrically by formamide extraction of the Coomassie Brilliant Blue R-250-stained subunit bands corresponding to the large and small subunits of Rubisco using calibration curves made with Rubisco purified from rice leaves (Makino et al. 1994a).

### Growth analysis

Plants were sampled at 21, 42 and 63 d after germination for rice and at 7, 21 and 35 d after germination for wheat. Leaf area was determined with a leaf area meter (AMM-8; Hayashi-denko, Tokyo, Japan), and leaf blades, leaf sheaths and roots were oven-dried at 80°C for more than a week, and their dry weight was measured. RGR, NAR, LAR, SLA, LWR and NUE for GR were calculated from total dry weight, leaf area, leaf weight and leaf-N content of the whole plant, using the respective equations as below.

$$\text{RGR} = (1/W)(\Delta W / \Delta t) = [\ln(W_2) - \ln(W_1)] / (t_2 - t_1) \quad (1)$$

where  $W_1$  and  $W_2$  are total dry weights of the whole plant including roots at times  $t_1$  and  $t_2$ .

$$\begin{aligned} \text{NAR} &= (1/L)(\Delta W / \Delta t) \\ &= (W_2 - W_1) / (t_2 - t_1) [\ln(L_2) - \ln(L_1)] / (t_2 - t_1) \end{aligned} \quad (2)$$

where  $L_1$  and  $L_2$  are total leaf areas of the whole plant at times  $t_1$  and  $t_2$ .

$$\text{LAR} = L/W = (L_1/W_1 + L_2/W_2) / 2 \quad (3)$$

RGR can be also expressed by multiplying NAR by LAR,

$$\text{RGR} = \text{NAR} \times \text{LAR} = (1/L)(\Delta W / \Delta t)(L/W) \quad (4)$$

In addition, LAR can be subdivided into LWR and SLA using the equation,

$$\text{LAR} = \text{LWR} \times \text{SLA} = (Lw/W)(L/Lw) \quad (5)$$

where  $Lw$  is total leaf weight of the whole plant.

NUE for GR was calculated by dividing the NAR by total leaf-N content per total leaf area of the whole plant ( $\text{Ln}/L$ ) using the equation,

$$\begin{aligned} \text{NUE for GR} &= \text{NAR} \times (L/L_n) = (1/L)(\Delta W/\Delta t)(L/L_n) \\ &= (1/L_n)(\Delta W/\Delta t) \end{aligned} \quad (6)$$

### Statistical analyses

Data are presented as the mean  $\pm$  SD. Tukey–Kramer's HSD test was performed with JMP (SAS Institute Inc., Cary, NC, USA).

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