Effect of High Nitrogen Application on Two Components of Dark Respiration in a Rice Cultivar Takanari

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ABSTRACT : Plant growth and the two components of respiration, growth and maintenance, were compared between low and high nitrogen applications in hydroponic culture on a high-yielding rice cultivar 'Takanari' (*Oryza sativa* L.). Grain yield decreased by high nitrogen application, and thus this cultivar has low adaptability to nitrogen. Growth efficiency (GE) and net assimilation rate (NAR) were lower in the high-nitrogen plot. The maintenance coefficient (*m*) and growth coefficient (*g*) of dark respiration were 0.0111 d⁻¹ and 0.196 in the low-nitrogen plot and 0.0166 d⁻¹ and 0.237 in the high-nitrogen plot, respectively. Thus, high nitrogen application increased both *g* and *m*. Calculated R_m (maintenance respiration rate) was 70 and 90% of total respiration rate at heading, respectively. The significance of nitrogen adaptability and *g* was discussed.

Key words : crop growth rate, dark respiration, growth efficiency, maintenance, nitrogen, photosynthesis, rice.

The modification of plant respiration has been considered as a possible means of improving crop productivity (Evans, 1993). Growth efficiency (GE) reflects the contribution of dark respiration to plant productivity (Tanaka and Yamaguchi, 1968). GE is the ratio of crop growth rate (CGR, $g\ m^{-2}\ d^{-1}$) to gross photosynthetic rate (P_g, $g\ m^{-2}\ d^{-1}$). The GE remains between 0.8 and 0.6 during vegetative growth periods in rice, but sharply decreases thereafter (Tanaka and Yamaguchi, 1968). This decrease in GE could be due to either an increase in the ratio of dark respiration to gross photosynthesis or to a decrease in photosynthetic rate.

Dark respiration rate (R, $g\ m^{-2}\ d^{-1}$) consists of two components: the growth (R_g) and maintenance (R_m) respiration rates (McCree, 1970). R_m is related to biomass, and R_g to CGR or P_g (Amthor, 1989):

$$R = R_m + R_g = mW + gCGR \qquad (1)$$

where W is plant biomass or total dry weight ($g\ m^{-2}$), and *m* and *g* are coefficients of maintenance (d^{-1}) and growth (dimensionless), respectively. Actually, *m* and *g* mean specific maintenance respiration rate ($g\ g^{-1}\ d^{-1}$) and the ratio of R_g/CGR, respectively. Several reports suggest that lowering R_m leads to a substantial increase in yield (Sheehy et al., 1979; McCree, 1988), whereas the efficiency of the synthetic processes is difficult to improve (Penning de Vries, 1974).

Increased nitrogen absorption is necessary to increase photosynthetic rate or crop production. However, respiration rate increases with nitrogen concentration (Osada, 1966). Furthermore, excess nitrogen application reduces yield in some rice cultivars (Takeda and Kumura, 1959). A cultivar of which yield increases with nitrogen application is defined highly adaptable to nitrogen (Osada, 1966). The cultivar with low nitrogen adaptability responds to excess nitrogen by overgrowing during the vegetative growth period, resulting in an increased respiration rate and a decreased growth rate during the reproductive growth period (Takeda, 1961). Nevertheless, the effects of nitrogen levels on the two components of dark respiration are not yet well known.

Here, the effect of nitrogen on plant growth and the two components of dark respiration were studied with the recently-developed Japanese high-yielding rice cultivar 'Takanari'.

MATERIALS AND METHODS

Rice seedlings of cv. Takanari, 26-day-old, were transplanted to an experimental field at the University of Tokyo on 17 May, 1998. One seedling was planted per hill (20 × 15 cm) at a density of 35 hills m^{-2} in water culture baths (3.0 × 3.4 × 0.5 m). The baths were filled with 30 cm -deep river gravel (each pebble was less than 3 cm in diameter) to hold

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the plants.

Nitrogen was initially applied in the form of $(NH_4)_2SO_4$ at concentrations of either 5 or 40 mg L^{-1}. These concentrations were doubled to 10 mg L^{-1} (low N) or 80 mg L^{-1} (high N) at 35 days after transplanting and were maintained until harvest. Other nutrients were added as described by Lee and Akita (2000). Nutrient solution (approximate pH 5) of 1000 L per plot and renewed every week.

To measure plant dry weight, 6 to 12 plants were sampled and separated into roots, stems (including leaf sheaths), leaf blades, panicles, and dead organs. Leaf area was measured only using the green parts of leaves (AAM-7, Hayashi Denko Inc., Japan), dried for at least 3 days at 80°C. Total nitrogen was measured by the semi-micro Kjeldahl method (VS-SA-1, Mitamura Riken Kogyo Inc., Japan).

To measure dark respiration, rice plants were put in measuring chambers and placed in a growth cabinet that was temperature-regulated at 25°C and supplied with CO_2-free air produced by passing the air through soda lime. Whole-plant respiration was measured continuously from around sunset to the next morning using an open-flow systemic infrared gas analyzer (ZRH, Fuji Denki Co., Japan). We used a flow controller that can handle four lines to measure three samples per analyzer per time course. Of the four lines, one was used to provide CO_2-free air to calibrate disturbance of the zero point in the analyzer during measurement. Data from two hours after sunset to sunrise the next day were used to calculate specific dark respiration rate (per unit dry weight), excluding the weight of dead organs. A coefficient (30/44) was used to convert respiration values from CO_2 to CH_2O.

CGR (g m^{-2} d^{-1}) was calculated from the average rate of increase of dry weight (including dead organs) between two sampling periods. R (g m^{-2} d^{-1}) was calculated from the average of specific dark respiration rate at 25°C and the average daily temperature between the two sampling periods, assuming a temperature coefficient (Q_{10}) of 1.8 (Lee and Akita, 2000). The daily solar radiation and mean temperature data for Tokyo in 1998 were used. P_g was calculated as the sum of CGR and R. The net assimilation rate (NAR, g $m^{-2}d^{-1}$) was calculated as CGR/leaf area index

(LAI). GE was calculated as CGR/P_g (Tanaka and Yamaguchi, 1968).

Several methods have been used to evaluate g and m (Amthor, 1989). In this paper, the coefficients were obtained by a regression method using the following equation derived from Equation (1):

$$R/W = m + g CGR/W \tag{2}$$

where TDW (g m^{-2}) is the average total dry weight excluding dead organs, because R_m is a measure of living organs. The slope of each regression line indicates g and the y-intercept indicates m. R_m and R_g were calculated using equation (1) and the values of m and g.

RESULTS

Rice plants cv. Takanari in high-N plot had greater dry weight increase than those in low-N plot during the vegetative growth period, but less than low-N plants during ripening (Table 1). At the heading stage, the average dry weight was 890 and 1415 g m^{-2} in the low- and high-N plots, respectively. In contrast, the increase in dry weight during ripening was 307 and 94 g m^{-2}, or 34.5% and 6.6% of the dry weight at heading, in the low- and high-N plots, respectively. Panicle dry weight at harvest was 510 and 270 g m^{-2} in the low- and high-N plots, respectively. Thus, the grain yield in the high-N plot was about half that of the low-N plot. Heading occurred about 10 days later in the high-N than in the low-N plot (Fig. 1).

The amount of absorbed nitrogen at heading was 12.95 and 43.78 g m^{-2} in the low- and high-N plots, respectively (Table 1). However, the absorption after heading was negligible in the high-N plot, whereas in the low-N plot it was still 65% of the amount at heading. Thus, root activity in the high-N plot was probably very low during ripening.

LAI increased markedly under high-N conditions (Table 1). The maximum LAI in the low- and high-N plots was 3.9 and 12.1, respectively, and was reached at heading in the low-N plot and about 20 days before heading in the high-N plot. Thus, leaf area decreased earlier and faster in the high-N plot.

Table 1. Amount of absorbed nitrogen (g m^{-2}), leaf area index (LAI), total dry weight (TDW, g m^{-2}), and panicle dry weight (PDW, g m^{-2}) in low- and high-N plots at heading and harvest.

N level	At heading			At harvest			
	Absorbed N (g m^{-2})	LAI	TDW (g m^{-2})	Absorbed N (g m^{-2})	LAI	TDW (g m^{-2})	PDW (g m^{-2})
Low	12.95	3.9	890	21.38	2.5	1197	510
High	43.78[‡]	11.3[‡]	1416[‡]	44.70[‡]	5.4[‡]	1510[†]	270[‡]

[†] and [‡]significantly different in the same column at 0.05 and 0.01 probability, respectively.
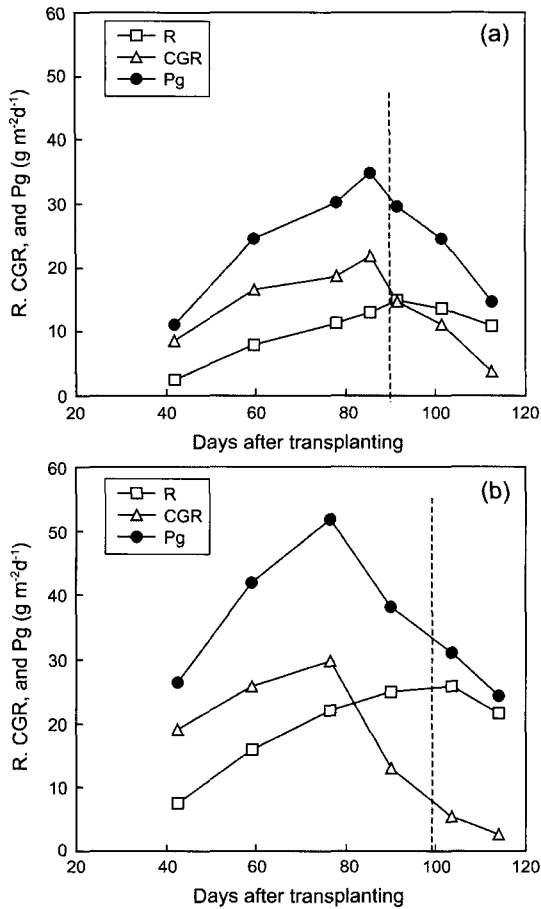
Fig. 1. Changes in the dark respiration rate (R), crop growth rate (CGR), and gross photosynthesis (P_g) in low- (A) and high-N (B) plots. Dashed line indicates the heading date.

Fig. 2. Changes in growth efficiency (GE) and net assimilation rate (NAR) in low- and high-N plots. Arrow indicates the heading date.

Table 2. Maintenance (m) and growth (g) coefficients of dark respiration in low- N and high N plots.

N level	m (d^{-1})	g	$r^{2\dagger}$
Low	0.01108	0.196	0.937
High	0.01661	0.237	0.985

†coefficient of determination.

The maximum CGR reached 22 and 30 g m^{-2} d^{-1} in the low- and high-N plots, respectively (Fig. 1). Decreases in CGR and P_g began about 20 days before heading in the high-N plot. However, R varied similarly in each plot although R was higher in the high-N plot. At heading, the R value was 35% and 80% of P_g in the low- and high-N plots, respectively. Thus, the early, large reduction of P_g in the high-N plot caused the reduced growth rate during the late-growth period.

Growth efficiency was higher in the low-N plot throughout the growth period, although CGR in the high-N plot was higher in the early vegetative growth period (Fig. 2a). In the early vegetative growth period, GE was between 0.78 and 0.62, but it decreased sharply in the high-N plot about 20 days before heading. At heading, GE was 0.5 in the low-N plot compared with 0.2 in the high-N plot.

NAR was also lower in the high-N plot throughout the growth period (Fig. 2b). At heading, NAR was more than 4-fold higher in the low-N than in the high-N plot. Thus, high-N application caused reduction of NAR and GE from the early growth period onward.
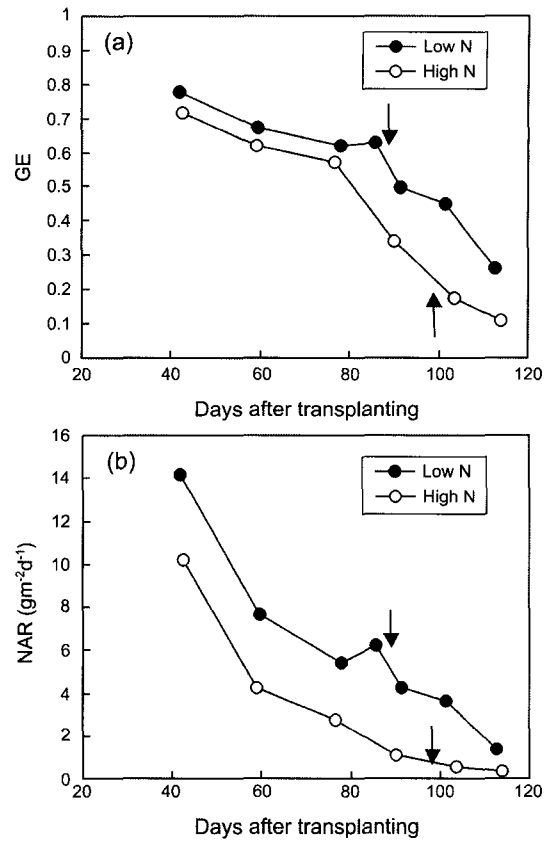
Interestingly, m and g increased with nitrogen application (Table 2). The value of m was 0.0111 and 0.0166 d^{-1}, and the value of g was 0.196 and 0.237, in the low- and high-N plots, respectively.

R_m and R_g were calculated using the values of m and g in Table 2 (Fig. 3). R_m gradually increased until harvest in the low-N plot but plateaued near the time of heading in the high-N plot. It reached about 70 and 90% of the calculated total respiration rate around heading in the low- and high-N plots, respectively, such that the ratio of R_m to the total respiration rate increased with growth. Thus, maintenance respiration explained most of R during ripening, especially in the high-N plot.
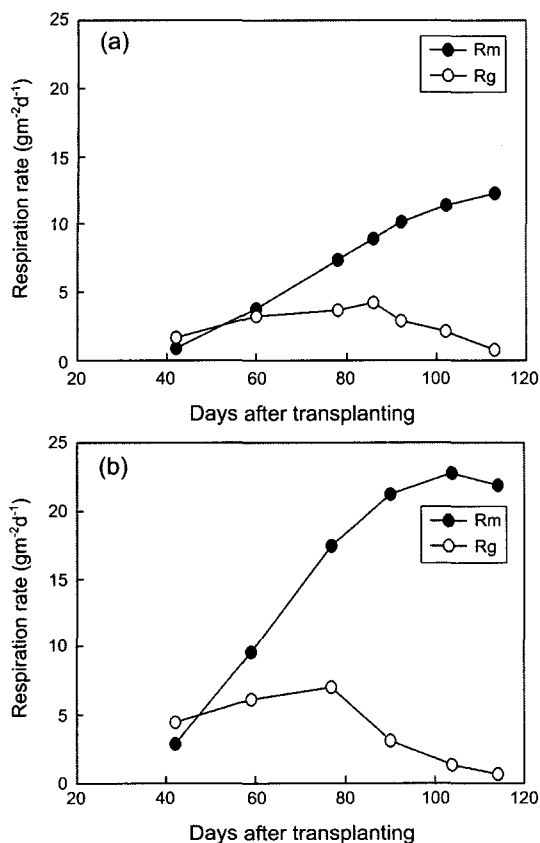
Fig. 3. Comparison of the rates of maintenance respiration (R_m) and growth respiration (R_g) in low-N (A) and high-N (B) plots. R_m and R_g were calculated using equation (1) and the coefficient values in table 2.

DISCUSSION

The rice cultivar 'Takanari' is a recently-developed, high-yielding semi-dwarf indica-type rice (Hiraoka *et al.*, 1992). It has a high assimilation ability and produces large numbers of spikelets when growth conditions are optimal (Xu *et al.*, 1997). In the field experiment, it had higher yield with high nitrogen application (Ishikawa *et al.*, 1999). However, grain yield of Takanari decreases significantly in high-N condition in this experiment (Table 1). Decreased fertilization and translocation of carbohydrates could decrease grain filling (Osada, 1966). This suggests that Takanari has low adaptability to increased nitrogen concentrations, probably because increased leaf shading in turn increases the proportion of R to P_g (Osada, 1966). In this study, decreasing photosynthetic rates led to an increased R proportion (Fig. 1). In addition, heavy nitrogen application also decreased GE and NAR from the early vegetative growth period when leaf shading did not occur (Fig. 2).

The pattern of variation in rice GE shown here is consistent with earlier reports (Tanaka and Yamaguchi, 1968;

Yamaguchi, 1978). Declines in GE during ripening have been shown to vary with growing conditions, and especially with the amount of nitrogen applied (Yamaguchi, 1978). We confirmed here that heavy nitrogen application negatively affects GE. To increase crop productivity under conditions of intense nitrogen application, it may be essential to increase nitrogen adaptability or nitrogen use efficiency in rice.

Hirota and Takeda (1978) observed $0.0092\sim0.0286$ d^{-1} of *m* and $0.187\sim0.539$ of *g* in rice. Our value of *m* and *g* was $0.0111\sim0.0166$ d^{-1} and $0.196\sim0.237$, respectively. The value of *m* and *g* varies according to plants and organs (Amthor, 1989).

It is generally accepted that *m* varies with growing conditions, while *g* does not (Penning de Vries, 1974). In sorghum (Stahl and McCree, 1988) and peas (Hole and Barnes, 1980), however, *g* decreases with increasing plant size and age. Our results also indicate that *g* varies with nitrogen level (Table 2). Heavy N application increases *m* by inducing plants to absorb more nitrogen and thus to increase protein content (McCree, 1983). Although the physiological mechanism for increased *g* due to heavy N application is unknown, it may be related to our finding that GE is lower in high-N plots, because *g* is negatively related to growth conversion efficiency (or true GE) (Thornley, 1970; Amthor, 1989). Therefore, when GE of whole-plant generally decreases during development (Tanaka and Yamaguchi, 1968; Yamaguchi, 1978), *g* of whole-plant should increase as growth proceeds, although this prediction remains to be proved.

The significance of *g* in crop productivity has rarely been discussed. It may derive from a premise that *g* does not vary but *m* does (Penning de Vries, 1974). The value of *m* is closely related to CGR (McCree and Kresovich, 1978) and decreases during development (Stahl and McCree, 1988). Heavier biomass, caused by a higher CGR, requires more photosynthates for R_m. The proportion of R_m to R increases with growth duration (Fig. 3). Decrease of photosynthesis further increases the R_m/R ratio. R_m has priority over R_g for photosynthate use (Amthor, 1994). Thus, plants may theoretically use the rest substrates, not during R_m, for growth and R_g. How much substrate is used for R_g or CGR is determined by *g* from Equation (3); lower *g* values induce higher CGR at the cost of R_g.

From equation (1), $1/g = CGR/R_g$ $\qquad\qquad$ (3)

As *g* decreases, the use efficiency of substrates to CGR increases. Therefore, discussion with *g* would be necessary to increase crop productivity. Here, we propose that higher plant productivity would be related to lower *g* as well as higher GE.

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