Planta (1997) 203: 390–398

Planta

Leaf photosynthesis, plant growth and nitrogen allocation in rice under different irradiances

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Received: 23 February 1997 / Accepted: 8 May 1997

Abstract. The photosynthetic rates and various components of photosynthesis including ribulose-1,5-bisphosphate carboxylase (Rubisco; EC 4.1.1.39), chlorophyll (Chl), cytochrome (Cyt) *f*, and coupling factor 1 (CF_1) contents, and sucrose-phosphate synthase (SPS; EC 2.4.1.14) activity were examined in young, fully expanded leaves of rice (*Oryza sativa* L.) grown hydroponically under two irradiances, namely, 1000 and 350 μmol quanta $\cdot m^{-2} \cdot s^{-1}$, at three N concentrations. The light-saturated rate of photosynthesis measured at 1800 μmol $\cdot m^{-2} \cdot s^{-1}$ was almost the same for a given leaf N content irrespective of growth irradiance. Similarly, Rubisco content and SPS activity were not different for the same leaf N content between irradiance treatments. In contrast, Chl content was significantly greater in the plants grown at 350 μmol $\cdot m^{-2} \cdot s^{-1}$, whereas Cyt *f* and CF_1 contents tended to be slightly smaller. However, these changes were not substantial, as shown by the fact that the light-limited rate of photosynthesis measured at 350 μmol $\cdot m^{-2} \cdot s^{-1}$ was the same or only a little higher in the plants grown at 350 μmol $\cdot m^{-2} \cdot s^{-1}$ and that CO_2-saturated photosynthesis did not differ between irradiance treatments. These results indicate that growth-irradiance-dependent changes in N partitioning in a leaf were far from optimal with respect to N-use efficiency of photosynthesis. In spite of the difference in growth irradiance, the relative growth rate of the whole plant did not differ between the treatments because there was an increase in the leaf area ratio in the low-irradiance-grown plants. This increase was associated with the preferential N-investment in leaf blades and the extremely low accumulation of starch and sucrose in leaf blades and sheaths, allowing a more efficient use of the fixed carbon. Thus, morphogenic responses at the whole-plant level may be more important for plants as an adaptation strategy to light environments than a response of N partitioning at the level of a single leaf.

Key words: Gas exchange (photosynthesis) – Growth analysis – Growth irradiance – Nitrogen partitioning (photosynthesis) – *Oryza* – Ribulose-1,5-bisphosphate carboxylase/oxygenase

Introduction

Leaf photosynthesis is strongly affected by the level of irradiance under which plants are grown. Generally, plants growing under high irradiance show high rates of photosynthesis at strong light intensities, and the plants growing under low irradiance give relatively high rates of photosynthesis at low light intensities. In addition, the light-saturation point also increases with increasing irradiance during growth (for reviews, see Boardman 1977; Björkman 1981). These responses of photosynthesis may be related to changes in leaf N content that are dependent on the light environment during plant growth (Evans 1987; Hirose and Werger 1987; Seemann et al. 1987; Terashima and Evans 1988; Hikosaka and Terashima 1996). Growth at higher irradiance is always associated with greater N content in a leaf, when N supply is adequate for growth.

However, several studies have reported that the response of leaf photosynthesis to growth irradiance is not only determined by a change in absolute N content in a leaf but also by changes in partitioning of N among photosynthetic components (for reviews, see Evans 1989; Terashima and Hikosaka 1995). For example, leaves developing under low irradiance show increased N allocation to chlorophyll (Chl), relative to electron-transport components and relative to ribulose-1,5-bis-

Abbreviations: CF_1 = coupling factor 1; Chl = chlorophyll; Cyt = cytochrome; LAR = leaf area ratio; *pCa* = ambient CO_2 partial pressure; *pCi* = intercellular CO_2 partial pressure; PPFD = photosynthetic photon flux density; RGR = relative growth rate; Rubisco = ribulose-1,5-bisphosphate carboxylase/oxygenase; SPS = sucrose-phosphate synthase

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phosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) protein (Evans 1987; Seemann et al. 1987; Terashima and Evans 1988; Hikosaka and Terashima 1996). Evans (1989) pointed out that such dependence of N partitioning on the conditions of growth irradiance is important for efficient photosynthesis. In addition, Hikosaka and Terashima (1995) extended his discussion and theoretically modelled an optimal N partitioning between the components related to light harvesting and those related to energy transduction, such as electron transport and CO_2 fixation, to realize maximum daily photosynthesis.

On the other hand, Lee and Whitemarsh (1989) reported that the potential adjustment of the activity and amount of thylakoid components by growth irradiance was quite small and concluded that the photosynthetic apparatus is relatively fixed irrespective of growth irradiance. Similarly, Lauerer et al. (1993) used transgenic tobacco plants with decreased Rubisco and reported that the plants clearly do not optimize N allocation at low irradiance even if they have adapted to low irradiance. According to them, the changes in N partitioning that are dependent on different light environments are too small to account for the optimization of N allocation. Thus, it remains unclear whether changes in N partitioning in a leaf are essential to explain a higher N-use efficiency of photosynthesis.

Irradiance also affects whole-plant growth at the morphological level. Generally, plants growing under low irradiance show increased plant length or stem elongation, decreased branching or tillering, and wider and thinner leaf development (Crookston et al. 1975; Vince-Prue 1977; Inada and Nishiyama 1987). Those researchers interpret the wider and thinner leaf development as an adaptive phenomenon to capture insufficient irradiance more efficiently. In addition, the ratio of leaf area development to plant-mass increment during growth increases with decreasing irradiance (Inada and Nishiyama 1987). Thus, these morphogenic responses are also important for high photosynthetic efficiency at the level of the whole plant.

In this study, we first elucidate how growth irradiance affects absolute N content and N partitioning in a leaf and discuss whether those changes in the N allocation that are dependent on the irradiance during growth are physiologically significant. We grew rice plants hydroponically under two growth irradiances of 350 and 1000 μmol $\cdot$ $m^{-2} \cdot s^{-1}$, at three different N levels, and compared the patterns of N allocation into several key components of each photosynthesis-limiting process. We measured Rubisco as a determinant for CO_2-limited photosynthesis (von Caemmerer and Farquhar 1981; Evans 1983), Chl as a light-harvesting component, cytochrome (Cyt) *f* and coupling factor 1 (CF_1) as rate-limiting factors for electron transport (Leong and Anderson 1984; Evans 1987; Price et al. 1995), and sucrose-phosphate synthase (SPS; EC 2.4.1.14) as a key enzyme during sucrose synthesis (Huber and Huber 1996). Second, we investigated changes in biomass allocation and in the rate of growth under different irradiances at the whole-plant level to get an overall picture of plant growth.

Materials and methods

Plant culture. Rice (*Oryza sativa* L. cv. Notohikari) plants were grown hydroponically in an environmentally controlled growth chamber (Makino et al. 1994). The chamber was first operated with a 14-h photoperiod, 25/20 °C day/night temperature, 60% relative humidity, and a photosynthetic photon flux density (PPFD) of 1000 μmol quanta $\cdot$ $m^{-2} \cdot s^{-1}$ at plant level during the daytime. Irradiance was provided by a combination of metal-halide lamps (Yoko DF; Toshiba, Tokyo, Japan) and high-output fluorescent lamps (FPR 96 EX-N/A; Panasonic, Tokyo, Japan). The basal nutrient solution was as previously described by Makino et al. (1988). From day 49 after germination, plants were grown at two irradiances, i.e., 350 and 1000 μmol quanta $\cdot$ $m^{-2} \cdot s^{-1}$, and N concentrations (mM) in the hydroponic solutions were 0.5 (0.25 mM NH_4NO_3), 2.0 (1.0 mM NH_4NO_3), and 8.0 (2.5 mM NH_4NO_3 plus 3.0 mM $NaNO_3$) for each irradiance treatment. These solutions were renewed once a week, and continuously aerated. Gas exchange, biochemical assays, and anatomical observations were carried out on young, fully expanded leaves of 70- to 80-d-old plants. Growth analyses of whole plant were done between 56- and 70-d-old plants.

Gas-exchange measurements. Gas exchange was determined with an open gas-exchange system using a temperature-controlled chamber equipped with two fans. The system was previously detailed by Makino et al. (1988). Differences in the partial pressures of CO_2 and H_2O entering and exiting the chamber were measured with an infrared gas analyzer (ASSA-1110; Horiba, Kyoto, Japan) and a dew-point hygrometer (model 911; EG&G, Natick, Mass., USA), respectively. Measurements were made at a leaf temperature of 25 °C, a PPFD of 350 or 1800 μmol quanta $\cdot$ $m^{-2} \cdot s^{-1}$, and a leaf-to-air vapor pressure difference of 1.0–1.2 kPa. The first measurement was made at an ambient CO_2 partial pressure (*pCa*) of 36 Pa to obtain the steady-state of the gas-exchange rate, and then *pCa* was varied. Carbon dioxide-saturated photosynthesis was measured above an intercellular CO_2 partial pressure (*pCi*) of 60 Pa. Gas-exchange parameters were calculated according to the equations of von Caemmerer and Farquhar (1981).

Biochemical assays. Total leaf N, Chl, Rubisco, Cyt *f*, and CF_1 contents of the leaf blade used for the gas-exchange measurements were determined as described by Makino et al. (1994). The leaf blade was homogenized in 50 mM Na-phosphate buffer (pH 7.0) containing 120 mM 2-mercaptoethanol, 2 mM iodoacetic acid and 5% (v/v) glycerol. The total leaf N and Chl contents were measured from part of this homogenate. To solubilize membrane-bound Rubisco, a Triton X-100 solution to a final concentration of 0.1% (v/v) was added to a portion of the leaf homogenate (Makino and Osmond 1991). The amount of Rubisco was determined spectrophotometrically after formamide extraction of Coomassie brilliant blue R-250-stained subunit bands separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A calibration curve was made with Rubisco purified from rice leaves. The remaining leaf homogenate was used for the determinations of Cyt *f* and CF_1. These were determined by rocket immunoelectrophoresis after solubilization with lithium dodecyl sulfate as described by Plumley and Schmidt (1983) with slight modification (Makino et al. 1994). Polyclonal-monospecific antibodies against Cyt *f* and the α and β subunits of CF_1 were used according to Hidema et al. (1991).

Sucrose-phosphate synthase activity was measured on a subsample of each treatment by the method of Huber et al. (1989), as described by Nakano et al. (1995). The assay was carried out at 25 °C under V_{max} substrate conditions.

Leaf-structural observations. The middle region of the leaf blade on the main stem of the plants grown at 2 mM N was used. An approximately 1-mm-thick cross section was fixed at 4 °C for 48 h with a solution containing 1.85% (v/v) formaldehyde, 5% (v/v) acetic acid, and 63% (v/v) ethanol. The fixed tissues were sequentially dehydrated with butanol, embedded in paraffin, sliced

into 10-μm sections, stretched onto glass slides coated with Vectabond Reagent, deparaffininzed with xylene and ethanol, rinsed, and stained with 0.05% (w/v) Toluidine Blue O, as described previously by Hayakawa et al. (1994). Pictures of the cross-sections of the blades were taken with an Olympus light microscope (model Vanox AHB-LB; Olympus, Tokyo, Japan).

Plant growth analysis. Eight plants per each treatment were sampled on days 56 and 70 after germination. These were harvested in the middle of the dayperiod. Leaf area was measured, and leaf blades, leaf sheaths, and roots were separately oven-dried at 80 °C for 3 d. Stems did not develop in any plants during the experimental period, and their dry weights were negligible. Relative growth rate (RGR) and leaf area ratio (LAR) were calculated from total dry weight and leaf area of the shoot.

Nitrogen and carbohydrate. For determination of total N (reduced-N) content, dried ground materials were digested with H_2SO_4-H_2O_2 at 260 °C. Total N content was determined with Nessler's reagent and a sample of the digestion solution after the addition of potassium sodium tartrate. The remaining dried materials were used for the determination of nitrate, sucrose, and starch. Nitrate was extracted with 80% (v/v) ethanol at 80 °C. After evaporation of the ethanol, the extract was distilled in the presence of Devarda's alloy by the microdiffusion method of Conway. Nitrate content was estimated by subtracting the ammonium content in the presence of Devarda's alloy from the content in the absence of Devarda's alloy. The ammonium contents were measured with Nessler's reagent. Sucrose was also extracted with 80% (v/v) ethanol at 80 °C. The sucrose content was determined by the method of Jones et al. (1977), as described by Nakano et al. (1995). Starch in the ethanol-insoluble fraction was extracted with 0.5 M KOH and neutralized with 1 M $HClO_4$. After removal of $KClO_4$, the starch was digested with amyloglucosidase (Nakano et al. 1995). The glucose content was determined by Somogyi-Nelson's method, and the amount of starch was calculated by multiplying its glucose content by 0.9.

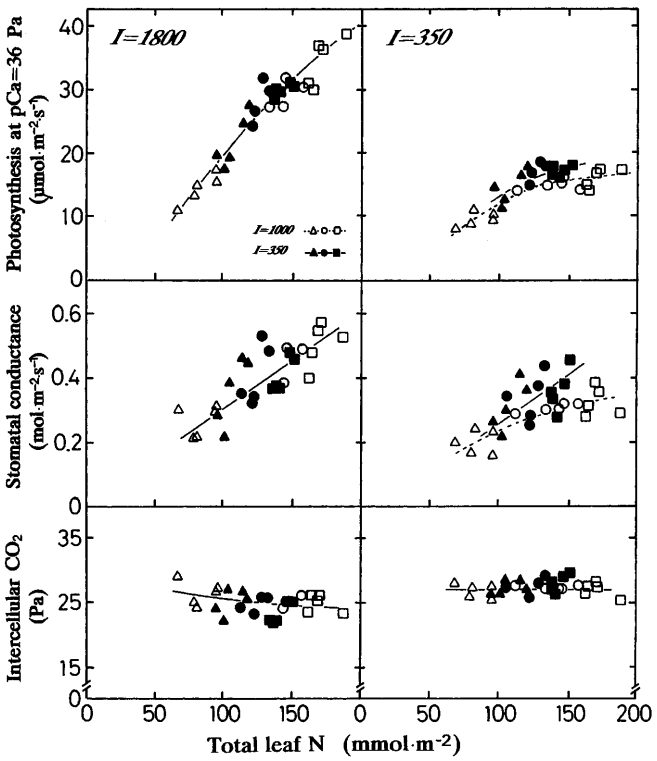

Fig. 1. Rate of photosynthesis at *pCa* = 36 Pa, stomatal conductance, and *pCi* versus total leaf N content. Measurements were made at a PPFD of 1800 μmol quanta · m^{-2} · s^{-1} (*left panel*) or 350 μmol quanta · m^{-2} · s^{-1} (*right panel*), a leaf temperature of 25 °C, and a leaf-to-air vapor pressure difference of 1.0–1.2 kPa. Plants were grown hydroponically under two irradiances of 1000 (*open symbols*) and 350 (*closed symbols*) μmol quanta · m^{-2} · s^{-1}, at N concentrations of 0.5 (*triangle*), 2.0 (*circle*), and 8.0 (*square*) mM

Results

Light-saturated photosynthesis at normal CO_2 (36 Pa) and total N content of leaves grown at three N concentrations under two irradiances are shown in Table 1. The effect of growth irradiance on photosynthesis strongly depended on the N-nutrition status. For example, when N supply was low (see 0.5 mM treatment), light-saturated photosynthesis in the low-irradiance-grown plants was higher than in the high-irradiance-grown plants. On the other hand, when N supply was sufficient (see 8.0 mM treatment), the rate of photosynthesis tended to be greater in the high-irradiance-grown plants. In addition, these responses of photosynthesis were similar to those of leaf N content.

Figure 1 shows the relationships between light-saturated (1800 μmol quanta · m^{-2} · s^{-1}) and light-limited (350 μmol quanta · m^{-2} · s^{-1}) photosynthesis and total leaf N. The light-saturated rate of photosynthesis was almost the same for any given leaf N content irrespective of growth irradiance. Similarly, stomatal conductance and *pCi* obtained at normal CO_2 (36 Pa) did not differ between the treatments. The light-limited rate of photosynthesis measured at 350 μmol · m^{-2} · s^{-1} was 30–50% lower than the light-saturated rate of photosynthesis, and tended to be only a little higher in the low-irradiance-grown plants than in the high-irradiance-grown plants. Although this was associated with a small

Table 1. Light-saturated photosynthesis at *pCa* = 36 Pa and leaf N in leaf blades of rice grown under two irradiances and three N concentrations. Values are means ± SE (*n* = 4–5)

N treatment	Growth irradiance (μmol quanta · m^{-2} · s^{-1})	Photosynthesis[a] (μmol CO_2 · m^{-2} · s^{-1})	Leaf N (mmol · m^{-2})
0.5 mM	1000	14.4 ± 2.0	82 ± 11
	350	21.9 ± 3.7	106 ± 9
2.0 mM	1000	28.9 ± 1.9	143 ± 9
	350	27.4 ± 2.6	122 ± 7
8.0 mM	1000	34.2 ± 3.6	170 ± 9
	350	30.1 ± 1.1	141 ± 6

[a]Measurements were made at a PPFD of 1800 μmol quanta · m^{-2} · s^{-1}, a leaf temperature of 25 °C, a *pCa* of 36 Pa, and a leaf-to-air vapor difference of 1.0–1.2 kPa

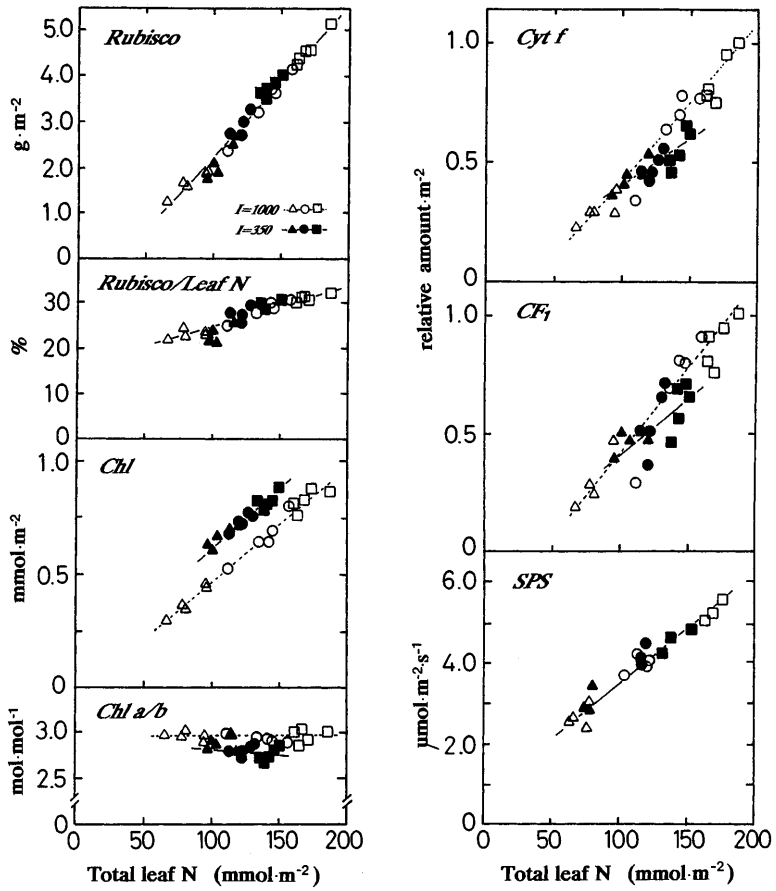

Fig. 2. Rubisco content, its ratio to leaf N, Chl content, Chl *a*/*b* ratio, Cyt *f* content, CF_1 content, and SPS activity versus total leaf N content. Symbols are the same as in Fig. 1

increased stomatal conductance, there was no difference in *pCi* between irradiance treatments. Thus, the response of the potential photosynthetic capacity to growth irradiance was almost accounted for by the response of leaf N to growth irradiance.

We next examined the relationships between several key photosynthetic enzymes and components and total leaf N content (Fig. 2). The amount of Rubisco was the same at any given leaf N content, and the ratio of Rubisco to total leaf N was 22–33%, irrespective of growth irradiance. This means that any shift in the N allocation between Rubisco and other components limiting photosynthesis was not dependent on the irradiance supplied in this experiment. In contrast, Chl content was greater in the low-irradiance-grown plants than in the high-irradiance-grown plants. On the other hand, Cyt *f* and CF_1 contents were slightly smaller in the low-irradiance-grown plants at high N content. However, CO_2-saturated photosynthesis did not differ between irradiance treatments at any leaf N content (Fig. 3). In addition, this small decrease in Cyt *f* and CF_1 contents was not reflected in light-limited photosynthesis. These results indicate that Cyt *f* and CF_1 are not necessarily rate-limiting factors for CO_2-saturated and light-limited photosynthesis. The activity of SPS did not differ between irradiance treatments.

Light micrographs of cross-sections of the leaf blades from the two irradiance treatments are shown in Fig. 4. The thickness of the blade in the low-irradiance-grown

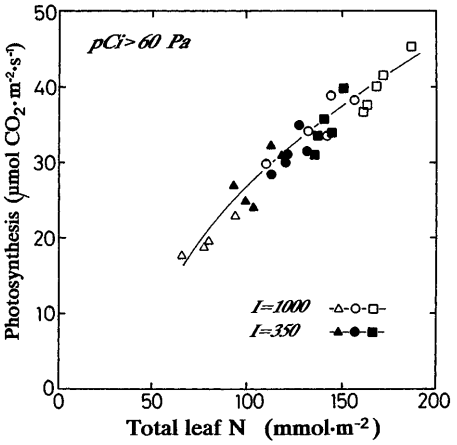

Fig. 3. Relationship between the rate of photosynthesis at $pCi > 60$ Pa and total leaf N content. Symbols are the same as in Fig. 1. Photosynthesis was measured at a PPFD of 1800 μmol quanta $\cdot$ $m^{-2} \cdot s^{-1}$, a leaf temperature of 25 °C, a *pCa* of 90–110 Pa, and a leaf-to-air vapor pressure difference of 1.0–1.2 kPa

plants was appreciably thinner, and the blade was especially constricted around bulliform cells. The average values of the leaf thickness around bulliform cells were 104 $\pm$ 10 μm and 81 $\pm$ 7 μm in the plants at 1000 and 350 μmol $\cdot$ $m^{-2} \cdot s^{-1}$, respectively ($\pm$SE, $n = 6$). In addition, relatively large bulliform cells with convex shapes were frequently observed for the low-irradiance-

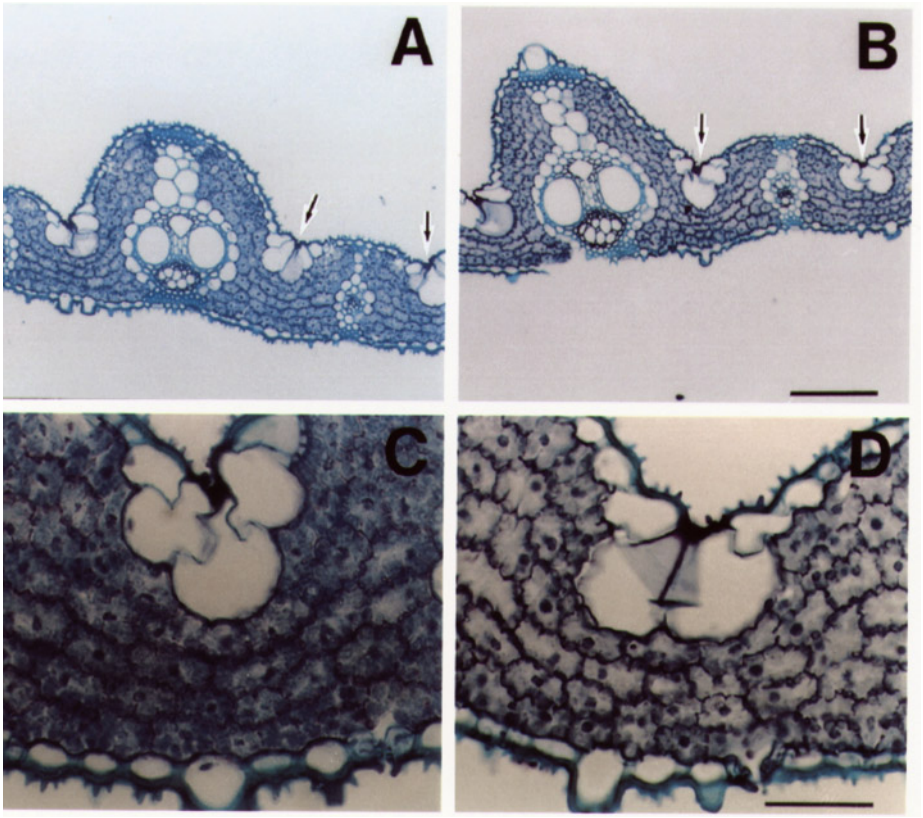

Fig. 4A–D. Light micrographs of cross-sections of the middle region of the leaf blades on the main stems from rice plants grown hydroponically under two irradiances of 1000 (**A**, **C**) and 350 (**B**, **D**) μmol quanta · $m^{-2} \cdot s^{-1}$ at an N concentration of 2.0 mM. The arrows indicate bulliform cells. Bars = 100 μm (upper panels), 30 μm (lower panels)

grown plants. Although its physiological significance is not known, these cells possibly play a role in light diffusion to neighboring mesophyll cells. The cell size of the mesophyll tissues was also slightly smaller in the low irradiance-grown plants, and this was particularly striking in the cells around bulliform cells. In dorsiventral leaves, the differentiation of the mesophyll tissues into palisade and spongy cells is important for light-use efficiency within a leaf (Terashima and Saeki 1983), and the palisade tissues are relatively thinner under low irradiance (Lee et al. 1990). However, since the mesophyll tissues of rice leaves consist only of armed-palisade cells, there is probably no predominantly structural difference between upper and lower cells depending on the growth irradiance.

Total plant dry weight at final harvest was significantly smaller at low irradiance than at high irradiance for all N treatments (Table 2). In addition, this difference due to growth irradiance also depended on N nutrition, and it increased with increasing N supply. The shoot/root ratio was higher in the low-irradiance-grown plants, and this was mainly due to the predominant decrease in the dry weights of the roots. By contrast, the total leaf area did not significantly differ between irradiance treatments, whereas the dry weight of the leaf blades (data not shown) was appreciably smaller in the low-irradiance plants.

The N investment in the leaf blades of the low-irradiance-grown plants was relatively great (Fig. 5). For all N treatments, the ratio of blade N to total plant N in the low-irradiance-grown plants was greater, and it increased with increasing N supply. Figure 6 shows the amount of nitrate pool in each organ. The nitrate pool was significantly greater in the low-irradiance-grown plants, and the amounts of nitrate stored in the leaf sheaths and the roots grown at 8 mM N were predominant, reaching 15% and 30% of total N in the respective organs. In contrast, the amount of nitrate in

Table 2. Plant mass and total leaf area of rice plants at final harvest (70 d after germination). Values are means ± SE (n = 8)

N treatment	Growth irradiance (μmol quanta · $m^{-2} \cdot s^{-1}$)	Shoot (g · $plant^{-1}$)	Root (g · $plant^{-1}$)	Shoot/Root ratio	Leaf area (cm^2 · $plant^{-1}$)
0.5 mM	1000	2.11 ± 0.12	1.50	1.41	141 ± 8
	350	1.63 ± 0.06	0.78	2.09	151 ± 8
2.0 mM	1000	2.34 ± 0.09	1.64	1.43	174 ± 7
	350	1.74 ± 0.12	0.67	2.60	196 ± 24
8.0 mM	1000	3.26 ± 0.31	1.81	1.80	250 ± 24
	350	2.09 ± 0.15	0.57	3.67	241 ± 15

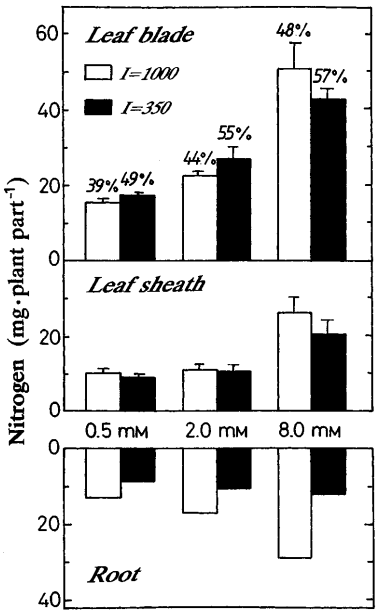

Fig. 5. Total N content in leaf blades, leaf sheaths, and roots from rice plants at final harvest (70 d after germination). Plants were grown hydroponically under two irradiances of 1000 (*open columns*) and 350 (*closed columns*) μmol quanta · m^{-2} · s^{-1} at N concentrations of 0.5, 2.0, and 8.0 mM. The percentage value above each column in the top panel shows the ratio of leaf blade-N to whole plant-N. The bar on each column indicates the SE (n = 8)

the leaf blades was very small irrespective of irradiance treatment, and it was less than 1% of total N even in the low-irradiance plants with high N supply. The amounts of sucrose and starch in the blades and sheaths are shown in Fig. 7. They were extremely small in the low-irradiance plants compared with those in the high-irradiance plants.

The growth rate of the whole plant under different irradiances was finally examined. The rate of growth was expressed as the RGR, which is defined as the dry weight increment per dry weight per d (Fig. 8). Surprisingly, in spite of a great difference in growth irradiance, the RGR

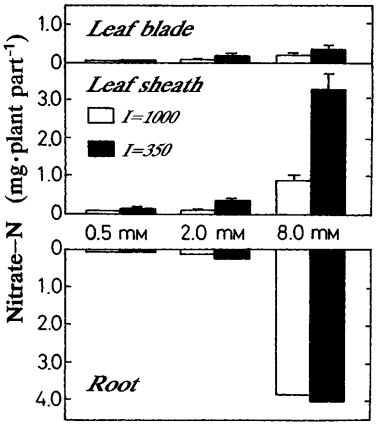

Fig. 6. Nitrate-N content in leaf blades, leaf sheaths, and roots from rice plants at final harvest (70 d after germination). Column symbols are the same as in Fig. 5. The bar on each column indicates the SE (n = 8)

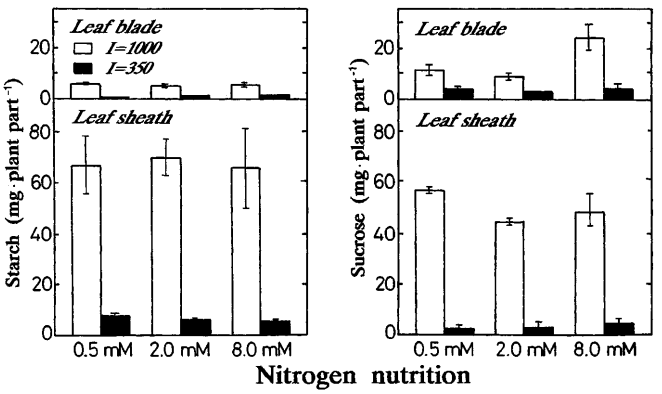

Fig. 7. Starch (*left panel*) and sucrose (*right panel*) contents in leaf blades and leaf sheaths from rice plants at final harvest (70 d after germination). Column symbols are the same as in Fig. 5. Plants were harvested in the middle of the day period. The bar on each column indicates the SE (n = 8)

of both treatments was almost the same. This means that the difference in plant mass at final harvest (Table 2) was caused by an initial difference in the growth rate within one week after the different irradiance supply. Since LAR was much greater in the low-irradiance-grown plants than in the high-irradiance-grown plants, the absence of difference in RGR between treatments was mainly due to the increase in investment in the leaf area in the plants grown under low irradiance. In addition, this increased LAR was associated with the preferential investment of N in the leaf blade (Fig. 5) and the extremely low accumulation of starch and sucrose, allowing a more efficient use of the fixed carbon. (Fig. 7).

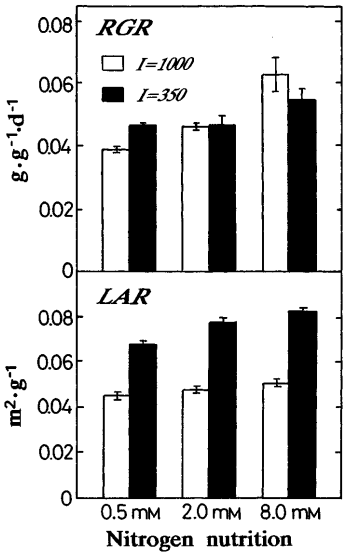

Fig. 8. Relative growth rate and LAR of plant shoots between 56 and 70 d after germination. Column symbols are the same as in Fig. 5. From day 49 after germination, plants were grown at two irradiance of 1000 and 350 μmol quanta · m^{-2} · s^{-1} and three N concentrations of 0.5, 2.0 and 3.0 mM. The bar on each column indicates the SE (n = 8)

Discussion

Physiological significance of changes in N partitioning in a leaf. Plants growing under low irradiance exhibit relatively increased N allocation to Chl and decreased electron-transport components and Rubisco protein relative to Chl, and show a slightly high rate of photosynthesis at low irradiance and a low rate of photosynthesis at high irradiance. These characteristics, including changes in the photosynthetic components in a leaf, have been considered as an acclimation to low irradiance (Evans 1989; Terashima and Hikosaka 1995). However, since these changes are frequently expressed on a Chl basis and since Chl content is most strongly affected by growth irradiance, such a change in Chl frequently makes it difficult to evaluate real changes in N partitioning among photosynthetic components depending on growth irradiance. Indeed, there are a few reports pointing out that the changes in the photosynthetic components are small (Lee and Whitmarsh 1989; Leuerer et al. 1993). Thus, it still remains unclear whether these changes are large enough to account for higher N-use efficiency of light-limited photosynthesis. In this study, therefore, we analyzed the changes in several key components of photosynthesis in relation to change in leaf N content by growth irradiance.

Ribulose-1,5-bisphosphate carboxylase is the most abundant leaf protein and its amount is clearly excessive under conditions of low irradiance (Sage et al. 1990; Quick et al. 1991; Leuerer et al. 1993). Therefore, if plants are potentially able to realize a higher N-use efficiency in low light environments, they should preferentially reduce the level of excess Rubisco. In our studies of rice plants, about 50% of Rubisco was calculated to be in excess for the observed rates of photosynthesis at 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (e.g. see Fig. 1). Nevertheless, the results in Fig. 2 clearly showed that Rubisco content at any given leaf N content did not decrease in the plants grown at 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$, compared with that in the plants grown at 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In agreement with this finding, neither was any difference due to growth irradiance found for the light-saturated rate of photosynthesis measured at normal CO_2 (Fig. 1). These findings were surprising, but similar results can be also found for the responses of thylakoid proteins to growth irradiance in pea (Evans 1987) and spinach (Terashima and Evans 1988). They reported that the proportion of N allocated to the thylakoids remained constant at 24–27% of total leaf N, irrespective of growth irradiance. Although changes in N partitioning within a thylakoid (more Chl and slightly less electron-transport components) were also found for our rice plants (Fig. 2), it remains questionable whether these changes are important enough to account for efficient photosynthesis under light-limited conditions. Many studies dealing with light-response curves of photosynthesis have shown that the slight increase in photosynthesis at low irradiance in low-irradiance-grown plants is not caused by an increase in apparent quantum yield but rather by lower rates of respiration (for review, see Boardman 1977; Björkman 1981). Terashima and Evans (1988) also showed that a slight increase in light-limited photosynthesis is mainly due to lower respiration for a given leaf N content. Thus, our results strongly cast doubt on whether changes in N partitioning among the photosynthetic components lead to a real greater N-use efficiency of light-limited photosynthesis and on whether they are essentially physiologically significant.

On the other hand, there have been some reports that plants grown under low irradiance contain decreased amounts of Rubisco relative to total leaf N (Seemann et al. 1987; Terashima and Evans 1988; Lauerer et al. 1993; Hikosaka and Terashima 1996). Regarding this discrepancy, there appear to be two possible reasons. One is the fact that a decrease in N content is always associated with a decrease in the ratio of Rubisco to total leaf N (Evans and Terashima 1988; Makino et al. 1992). According to our results, this was independent of the growth irradiance supplied in the experiments. This relative decrease in Rubisco is considered to be required to maintain the co-limitation balance between Rubisco capacity and other processes which limit photosynthesis at normal *pCa* because CO_2 partial pressure at the carboxylation site is relatively higher in leaves with less Rubisco (for a detailed discussion, see Evans and Terashima 1988). The other possible reason is the decrease in the ratio of Rubisco to total leaf N due to an increase in the nitrate pool under low irradiance. Such an increase means that N uptake exceeds the utilization of photosynthate for plant growth. In fact, the nitrate pool is not negligible for N economy in a leaf. For example, in tobacco plants, the ratio of nitrate to total leaf N reaches 10–15% even under high irradiance (Masle et al. 1993). In wheat, this ratio was estimated to be more than 25% (Dalling 1987). In our case with rice plants, however, although the nitrate pool was significantly greater in the low-irradiance-grown plants, it amounted to less than 1% of total N in the leaf blades, even in the plants grown at high N levels (Fig. 6). Therefore, the change in the nitrate pool did not affect the ratio of Rubisco to total leaf N. We thus consider that even if a relative decrease in Rubisco is found for other plants grown under low irradiance, this does not indicate an optimal N use in a leaf in low-irradiance environments.

Whole-plant physiology and growth under low irradiance. Although most of the dry weight of plants consists of photosynthetic products, many studies have shown that the photosynthetic rate per unit of leaf area does not necessarily reflect the rate of the whole-plant growth (Irvine 1975; Poorter and Remkes 1990; Gifford et al. 1984; Stitt and Schulze 1994). This means that morphology and biomass allocation at the whole-plant level as well as leaf photosynthesis are important for plant growth (Gifford et al. 1984; Stitt and Schulze 1994). Our results also showed there to be no difference in RGR between irradiance treatments, whereas the rate of photosynthesis should have been largely different under the different irradiances (Fig. 8). In our studies with rice plants, this was caused by an increase in LAR in the low-irradiance-grown plants. This indicates that the plants preferentially

invested their assimilate to enlarge the leaf area. Actually, in spite of the great difference in plant mass at final harvest between irradiance treatments, total leaf area was not significantly different (Table 2). This enlargement of leaf area relative to plant mass was also found for several other C_3 plants grown under conditions of shade (Inada and Nishiyama 1987). In addition, as shown in Fig. 5, the allocation of N into the leaf blades at the whole-plant level was relatively higher in the low-irradiance-grown plants. For the 0.5 and 2.0 mM N-treatments, the absolute amounts of N in the leaf blades were greater in the low-irradiance-grown plants. This may have been because rice plants genetically have high capacity of N assimilation even under conditions of low irradiance. This is supported by the extremely low nitrate content in rice (Fig. 6). Thus, rice plants had a relatively large leaf area and preferentially invested N in leaf blades to achieve efficient growth under low irradiance.

The rate of plant growth is also dependent on the photosynthate-use efficiency for growth. For example, accumulation of starch and sucrose can be an indicator of the inefficiency with which photosynthate is used for growth (Stitt and Schulze 1994). Some of these carbohydrates, such as the carbohydrates which are not remobilized during the night, may not make a contribution to growth, and thus be wasted. In our case with rice plants, the large accumulation of starch and sucrose was found in the high-irradiance-grown plants (Fig. 7). This was especially predominant in their leaf sheaths. Although significant diurnal changes in both starch and sucrose were found in the leaf blades, those in the leaf sheaths were very small (data not shown). Growth under conditions of 'excess' carbon in rice plants frequently leads to accumulation of carbohydrates in the leaf sheaths (Arashi and Eguchi 1954; Togari and Sato 1954; Nakano et al. 1995). On the other hand, accumulation was extremely low in both the blades and leaf sheaths of the low-irradiance-grown plants whereas they were sampled in the middle of the day. This was also probably related to efficient growth of the plants under low irradiance.

To summarize the results, preferential N-investment in the leaf blades and higher photosynthate-use efficiency, as well as enlargement of the leaf area may compensate for decreased photosynthesis per unit of leaf area at low irradiance. Consequently, the RGR of both treatments was almost the same, irrespective of growth irradiance. We conclude that these morphogenic responses, including biomass allocation at the whole-plant level, were more important as an adaptation strategy to light environments, and that changes in N partitioning among the photosynthetic components at the level of a single leaf were far from optimal with respect to N-use efficiency of photosynthesis at the low irradiance supplied.

This work was supported by Grants-in-Aid for Research for the Future from the Japan Society for the Promotion of Science (JSPS-RFTF 96L00604) and for the Integrated Research Program for the Use of Biotechnological Procedures for Plant Breeding from the Ministry of Agriculture, Forestry and Fisheries, Japan. We thank Nozomu Sakurai and Keiki Ishiyama for technical help and advice on light microscopy.

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