Differences between rice and wheat in RuBP regeneration capacity per unit of leaf nitrogen content

E Sudo, A Makino, T Mae

Department of Applied Plant Science, Graduate School of Agricultural Sciences, Tohoku University, Tsutsumidori-Amamiyamachi, Sendai 981-8555, Japan

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Introduction

A highly positive correlation between photosynthetic capacity and nitrogen content in leaves is often found in higher plant (Evans 1989). Light-saturated photosynthesis in air CO2 levels for a given leaf-N content is higher in wheat than in rice. This is caused by a greater Rubisco capacity in wheat (Makino et al. 1988). In this study, we found that light-saturated photosynthesis under elevated CO2 conditions is also higher in wheat than in rice (Fig. 1). This means that RuBP regeneration capacity is also greater in wheat. RuBP regeneration capacity can be limited by the electron transport capacity and/or Pi regeneration capacity for photophosphorylation during sucrose and starch synthesis (Farquhar and von Caemmerer 1982, Sharkey 1985). The factor, however, which determines the difference in RuBP regeneration capacity between the two species still remains unclear. In this study, we used young leaves of rice and wheat, and tried to identify the factor(s) for difference in RuBP regeneration between the two species. First, we investigated the differences in the nitrogen partitioning among soluble and insoluble proteins and trichloroacetic acid (TCA)-soluble fraction between the two species. we next examined the activities of several Calvin cycle enzymes such as Rubisco, NADP-G3PDH, PGA kinase. We also examined SPS activity as key enzyme during sucrose synthesis (Huber 1983, Stitt 1988) to evaluate Pi-regeneration capacity. In addition, we measured Chl content and Chl a/b ratio, Cyt f and CF1 contents as the key components of light-harvesting and electron transport system, respectively.

Materials and methods

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. Growth temperature for rice and wheat was maintained 26/20°C and 23/18°C (day/night), respectively. The basal hydroponic solution used for rice and wheat were described by Mae and Ohira (1981) and Makino and Osmond (1991) and their pH were adjusted to 5.5 and 4.5, respectively. Both plants were grown with three nitrogen concentrations (mM): 0.5 (0.25 mM NH4NO3),2.0 (1.0 mM NH4NO3), and 8.0 (2.5 mM NH4NO3 plus 3.0 mM NaNO3 for rice, 2.0 mM NH4NO3 plus 4.0 mM NaNO3 for wheat). All measurements were made on young, fully expanded leaves of 66- to 80-d-old plants in rice, 35- to 45-d-old plants in wheat.

Chl, total leaf N, Rubisco contents were determined according to Makino et al. (1994). The supernatant of the homogenate was used for the measurement of soluble-N and soluble protein-N. Soluble protein-N was measured using TCA-precipitate of the supernatant. Insoluble-N and TCA soluble-N was determined by means of subtraction soluble-N from total
leaf N, soluble protein-N from soluble-N, respectively. All nitrogen content was determined with Nessler’s reagent after Kjeldahl digestion (Makino et al. 1994). To determine CF1 contents, a portion of the homogenate was filtered with four layers of cheesecloth and centrifuged. The pellet containing thylakoid membrane was suspended in 50 mM Tris/HCl buffer (pH 7.2) and treated with a lithium dodecylsulfate solution (final concentration was 2.6% [w/v]) at 100°C for 90 s. The supernatant was analyzed by SDS-PAGE. BSA was used for making calibration curve. CF1 contents was determined spectrophotometrically by formamide extraction of Coomassie Brilliant Blue R-250 on its α and β subunits. Cyt f content was determined from the difference between the hydroquinone-reduced and the ferricyanide-oxidized spectra of the thylakoid membrane according to Evans and Terashima (1987). Rubisco activity was measured spectrophotometrically by coupling 3-phosphoglyceric acid formation with NADH oxidation at 25°C as described in Nakano et al. (2000). NADP-G3PDH, PGA kinase and cpFBPase activities were also measured spectrophotometrically at 25°C. NADP-G3PDH activity was determined according to Makino et al. (1994) with some modification. PGA kinase activity was measured as described in Makino et al. (1983). CpFBPase activity was determined according to Sharkey et al. (1991) with some modification. SPS activity was assayed according to Nakano et al. (1995) with some modification and the activity was calculated from the Michaelis-Menten’s equation by the methods of Wilkinson (1965). UDP-G concentration in the reaction mixture was varied 2 mM, 5 mM, 10 mM, 50 mM (final concentration).

**Results and Discussion**

The nitrogen partitioning among soluble and insoluble proteins and TCA-soluble fraction in rice and wheat leaves were examined. The ratio of insoluble-N, soluble protein-N and TCA soluble-N to total leaf N content did not differ significantly between the two species.

Activities of several Calvin cycle enzymes, Rubisco, NADP-G3PDH, PGA kinase and cpFBPase in rice and wheat leaves were determined. Although the amount of Rubisco protein was not different between the two species, Rubisco activity per unit of leaf nitrogen was higher in wheat. These were previously found by Makino et al. (1988). Furthermore, NADP-G3PDH, PGA kinase and cpFBPase activities were also higher in wheat. Among them cpFBPase activity was largely higher in wheat. Thus, all Calvin cycle enzymes examined here are higher. Generally, however, the potential activity for Calvin cycle enzymes was considered to be excessive for photosynthesis (Koßmann et al. 1994, Price et al. 1995a). Therefore, the Calvin cycle enzyme examined here did not limit RuBP regeneration in both species.

Although SPS is a key enzyme during Pi regeneration, the Vmax activity for UDP-G was almost the same between the two species and its Km value was a little lower in rice. It is suggested that in vivo SPS activity is greater in rice despite of lower RuBP regeneration capacity in rice. Thus, we conclude that SPS activity does not determine the difference in RuBP regeneration between the two species.
Chl content and Chl a/b ratio did not differ. This indicated light harvesting capacity in the two species was not different. Cyt f content was greater in wheat than in rice, whereas coupling factor 1 content was greater in rice. Among them, Cyt f content was most highly correlated with CO₂-saturated photosynthesis, irrespective of the two species. In addition, the regression line between Cyt f content and CO₂-saturated photosynthesis in both species fell on the same line (Fig. 2). This results, thus, suggest that higher RuBP regeneration capacity in wheat leaves is caused by a greater Cyt f content. Cyt f content was highly correlated with the whole-chain electron transport rate (Evans 1987, Terashima and Evans 1988) and O₂ evolution rate (Terashima and Evans 1988). In the transgenic tobacco with reduced amounts of Cyt bo/f complex, electron transport rate declined (Price et al. 1995b). Furthermore, Cyt f content was also highly correlated with photosynthetic rate at ambient CO₂ condition and RuBP pool size (Price et al. 1998). Makino et al. (1997) found that in the transgenic rice plants with reduced amount of Rubisco, the photosynthetic rate at elevated CO₂ level was higher than in the wild type rice because leaf nitrogen were reallocated from decreased Rubisco into Cyt f and CF₁ in the antisense rice. These results including ours indicate that Cyt f contents affect the electron transport rate and photosynthetic rate at elevated CO₂ level, that result in the RuBP regeneration rate.

Fig. 1 (left) Photosynthesis at pCi = 20 Pa (A₂₀) and pCi > 60 Pa (A₆₀), and the ratio of A₆₀/A₂₀. Data were from Makino (unpublished).

Fig. 2 (right) The relationship between photosynthesis at pCi > 60 Pa and Cyt f content in rice and wheat.
References

Evans JR (1989) Oecologia 78, 9-19
549-587. Springer Verlag, Berlin.
Huber SC (1983) Plant Physiology 71, 818-821
27, 167-173
Australian Journal of Plant Physiology 25, 445-452
Wilkinson GN (1961) Biochemical Journal 80, 324-332