Screening of High k[cat] Rubisco among Poaceae for Improvement of Photosynthetic CO[2] Assimilation in Rice

Ishikawa, Chie
Hatanaka, Tomoko
Misoo, Shuji
Fukayama, Hiroshi

(Citation)
Plant Production Science,12(3):345-350

(Issue Date)
2009

(Resource Type)
journal article

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/90002851

Plant Prod. Sci. 12(3) : 345—350 (2009)

Screening of High k_{cat} Rubisco among Poaceae for Improvement of Photosynthetic CO_2 Assimilation in Rice

Chie Ishikawa, Tomoko Hatanaka, Shuji Misoo and Hiroshi Fukayama

(Graduate School of Agricultural Science, Kobe University, Nada-ku, Kobe 657-8501, Japan)

Abstract : The activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a major limitation of photosynthetic CO_2 assimilation in C_3 plants. In order to find useful Rubisco for improvement of photosynthesis in rice under elevated CO_2, we analyzed the catalytic turnover rate (k_{cat}) of Rubisco in Poaceae including C_3 alpine plants, C_3 cold-resistant plants and C_4 plants. Rubisco in these plants showed 1.1- to 2.8-fold higher k_{cat} than that in rice. However, the most of high k_{cat} Rubisco also showed a higher km for CO_2 (Kc) than that of rice, indicating that increase in k_{cat} led to decrease in the affinity for CO_2. Rubisco in *Festuca ovina, Phleum pratense* and *Sorghum bicolor* showed relatively high k_{cat} to Kc. Although the k_{cat} of Rubisco in *F. ovina* and *P. pratense* was not so high (1.5-1.6 fold relative to rice), the Kc was comparable to that in rice and the amino acid sequence of *RbcL* shared higher identity to that in rice than that in *S. bicolor*. By contrast, Rubisco of *S. bicolor* showed considerably high k_{cat} (2.5-fold relative to rice), which is considered to be the most important factor for improvement of photosynthesis. In our estimation, the expression of high k_{cat} Rubisco of *F. ovina* and *S. bicolor* in rice could significantly enhance CO_2 assimilation at Ci of 50 Pa, the level assumed to be reached by the middle of this century.

Key words : k_{cat}, Km, *Oryza sativa* L., Photosynthesis, Rice, Rubisco.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a key enzyme of photosynthetic CO_2 fixation. Photosynthesis at present atmospheric CO_2 level in C_3 plants, including rice, is considered to be largely limited by the activity of this enzyme, because of its low rate of catalysis compared to the other enzymes (Makino et al. 1983; 1985; Evans, 1986). In addition, Rubisco also catalyzes competing oxygenase reaction that initiates a wasteful photorespiratory pathway leading to the loss of fixed carbon. Because of these defective enzymatic properties, C_3 plants invest 15 to 35% of total leaf nitrogen in Rubisco (Evans, 1989; Makino et al., 1992).

Rubisco in C_3 plants working at a low CO_2 concentration has been evolved to acquire high affinity for substrate CO_2 rather than high k_{cat} in order to suppress photorespiration. In particular, Rubisco of non-green algae such as *Galdieria* and *Griffithsia* are more specific to CO_2 than higher plant Rubisco (Whitney et al., 2001). However, Rubisco showing high specificity for CO_2 often shows a low k_{cat} in carboxylase reaction. In addition, it is difficult to express functional Rubisco from these genetically distant organisms in the chloroplast of higher plants (Whitney et al., 2001). Considering the recent global environmental changes, the atmospheric CO_2 concentration is rapidly increasing and is estimated to reach 60 Pa by the middle of this century. Under this elevated CO_2 condition, Rubisco no longer limits the photosynthesis and high k_{cat} will be an important factor for Rubisco to increase the photosynthetic efficiency rather than the specificity for CO_2.

There are significant natural variations in k_{cat} of Rubisco among higher plants (Seemann et al., 1984; Sage, 2002). The C_3 plants from cool-habitat and C_4 plants showed higher k_{cat} of Rubisco than C_3 plants from warm-habitat, including rice (Sage, 2002). In *Oryza* genus, the relatively high specific activities were found in Rubisco from CC genome (Makino et al., 1987), whereas these activities were much lower than that in C_3 plants from cool-habitat or C_4 plants (Sage, 2002). In order to find the efficient high k_{cat} Rubisco for the enhancement of rice photosynthesis, it is necessary to analyze in detail the kinetic properties of Rubisco in genetic resources other than *Oryza* genus.

Rubisco from higher plants is composed of eight large subunits (encoded by the chloroplast *RbcL*) and eight small subunits (encoded by a family of nuclear *RbcS*) in a hexadecameric structure. Most of the important amino acids for catalysis exist in *RbcL* (Andersson and Backlund, 2008). The specific activity of Rubisco showed maternal inheritance and was not associated with nuclear genes (Li et al., 1983; Evans and Austin, 1986), suggesting that *RbcL* is the major

---

Received 18 December 2008. Accepted 19 January 2009. Corresponding author: H. Fukayama (fukayama@people.kobe-u.ac.jp, fax +81-78-803-5820). This research was supported in part by a Grant-in Aid for Scientific Research (No. 20580014) from the Ministry of Education, Science, Sports and Culture, Japan.

Abbreviations : Ci, intercellular CO_2 partial pressure; Kc, the Michaelis constant for CO_2; k_{cat}, catalytic turnover rate; *RbcL*, Rubisco large subunit gene; *RbcS*, Rubisco small subunit gene; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate.

determinant of k_{cat} of Rubisco. From these view points, it is considered that one of the best ways to improve the photosynthetic capacity of rice in the near future atmospheric conditions is to find high k_{cat} Rubisco with high amino acid sequence similarity to rice Rubisco and substitute rice *RbcL* with its *RbcL* using genetic recombination technology.

In this study, we analyzed the kinetic properties of Rubisco among Poaceae including C_3 alpine plants, C_3 cold-resistant plants and C_4 plants. In addition, we determined the sequences of *RbcL* and made a comparison of the deduced amino acid sequences among higher plants. Finally, the estimation of CO_2 assimilation rate was carried out to elucidate whether high k_{cat} Rubisco found in this study could enhance the photosynthetic performance of rice under elevated CO_2.

Materials and Methods

1. Plant materials

Rice (*Oryza sativa* L. cv. Nipponbare), C_3 alpine plants, C_3 cold-resistant plants and C_4 plants of Poaceae were grown and sampled at optimum conditions to obtain well developed leaves. Rice was planted on a paddy soil and grown in the greenhouse. Upper most fully expanded leaves were sampled in the middle of July 2007. The mature leaves of C_3 alpine plants, *Anthoxanthum japonicum* Hack., *Calamagrostis longiseta* Hack., *Festuca ovina* L. and *Phleum alpinum* L. were sampled in early June 2007 at the Botanical Garden of the Hakusan Alpine Plants Research Group (Hakusan city, Japan). The C_3 cold-resistant plants, *Dactylis glomerata* L. cv. Aikappu (orchard grass), *Poa arctica* R. Br. ssp. *lanata*, *Phleum pratense* L. cv. Natsusakari (timothy) and *Secale cereale* L. cv. King Lye (rye) were planted on a commercial culture soil in late September, because they cannot grow well during summer under natural conditions. The mature leaves of these plants were sampled in the middle of December 2007. The C_4 plants, *Zea mays* L. cv. Golden×Bantam (maize), *Sorghum bicolor* L. Moench. cv. Tentaka (sorghum), *Panicum maximum* Jacq. cv. Natsuyutaka (guinea grass) were planted on a commercial culture soil and grown under natural condition. The mature leaves were sampled in the middle of July 2007. *Pennisetum purpureum* Schumach cv. Mericlone (napier grass) was grown in the experimental field of Kobe University and the mature leaves were sampled in the middle of July 2007. The mature leaves of *Eleusine indica* (L.) Gaertn., *Miscanthus sinensis* Anderss. and *Setaria viridis* P. Beauv. were sampled in the campus of Kobe University in the middle of September 2007. All of the leaves were collected at around 1100 in sunny days and immediately frozen in liquid nitrogen. Samples were stored in a −80°C freezer until use. All cultivated plants were watered and fertilized as needed.

2. Determination of Rubisco activity and catalytic site

About 2 cm^2 of sampled leaf tissues (in case of rice, 1 cm^2 of leaf tissues were used) were rapidly homogenized in 1 mL of extraction buffer (100 mM Bicine-NaOH, 1 mM EDTA, 5 mM $MgCl_2$, 2 mM NaH_2PO_4, 0.4% (w/v) BSA, 5 mM DTT, 4 mM amino-n-caproic acid, 0.8 mM benzamidine, pH 8.0) using a chilled motor and pestle with a small amount of quartz sand. The homogenate was then centrifuged at 15,000 *g* for 2 min at 4°C. The supernatant was used for the determination of Rubisco activity and Rubisco catalytic site.

Rubisco activity was determined at 28°C using [^{14}C] $NaHCO_3$ by assaying the incorporation of ^{14}C into acid-stable products, as described by Kubien et al. (2003) with some modifications. Rubisco in the extract was activated by pre-incubation with 15 mM $MgCl_2$ and 10 mM $NaHCO_3$ on ice for 15–20 min. The reaction was started by the addition of activated Rubisco to the reaction mixture containing 100 mM Bicine-NaOH, 20 mM $MgCl_2$, 1 mM EDTA, 5 mM DTT, 15 mM $NaH^{14}CO_3$ (specific activity, 3.7 MBq $mmol^{-1}$) and 0.4 mM RuBP, pH 8.2. After 1 min, 1/2 vol. of formic acid was added to the reaction solution to stop the reaction. The acidified reaction mixes were dried and acid-stable ^{14}C was measured by liquid scintillation.

Rubisco catalytic site concentrations were determined by measuring the stoichiometric binding of [^{14}C] carboxy-D-arabinitol-1,5-bisphosphate (CABP) to Rubisco, based on the method of Butz and Sharkey (1989) with some modifications. Rubisco in the extracts was incubated with the buffer containing 50 mM Bicine-NaOH, 1 mM EDTA, 20 mM $MgCl_2$, 15 mM $NaHCO_3$ and 0.02 mM ^{14}C-CABP (specific activity, 1.85 GBq $mmol^{-1}$), pH 7.8 at room temperature for 45 min to bind ^{14}C-CABP to Rubisco catalytic sites. Rubisco was precipitated with casein (2.0 mg mL^{-1}) as carrier by adding PEG 4000 and $MgCl_2$ to final concentration of 20% (w/v) and 25 mM, respectively. After 30 min, the solution was centrifuged at 15,000 *g* for 15 min. The pellet was washed three times with washing buffer containing 50 mM Bicine, 15 mM $MgCl_2$, 1 mM EDTA and 20% PEG4000, pH 7.8. Only at the first washing step, the pellet was thoroughly resuspended using a sonicator. The ^{14}C retained within the pellet was measured by liquid scintillation. The k_{cat} of Rubisco (mol mol^{-1} s^{-1}) was calculated as the ratio of *in vitro* Rubisco activity to Rubisco catalytic sites. For determination of Km for CO_2 of Rubisco (Kc), Rubisco activities were measured at six different $NaH^{14}CO_3$ concentrations (0.5-15 mM) as mentioned above and Kc was calculated from the Hanes-Woolf plot ([S]/v~[S] plot).

3. Analysis of partial RbcL sequence

Genomic DNA was extracted from leaf blades and purified by the CTAB method (Murray and Tompson, 1980). Partial fragments of *RbcL* were

Table 1. k_{cat} of Rubisco from a variety of C_3 and C_4 plants in Poaceae.

Species	k_{cat} (mol mol^{-1} s^{-1})	Fold (relative to rice)
Oryza sativa	1.39 ± 0.01	–
C_3 alpine plants		
Anthoxanthum japonicum	1.58 ± 0.05**	1.1
Calamagrostis longiseta	1.92 ± 0.11**	1.4
Festuca ovina	2.05 ± 0.07**	1.5
Phleum alpinum	2.22 ± 0.11**	1.6
C_3 cold-resistant plants		
Bromus inermis	1.73 ± 0.08**	1.2
Dactylis glomerata	1.59 ± 0.04**	1.1
Phleum pratense	2.18 ± 0.13**	1.6
Poa arctica	1.93 ± 0.04**	1.4
Secale cereale	1.65 ± 0.09*	1.2
C_4 plants		
Eleusine indica	2.73 ± 0.09**	2.0
Miscanthus sinensis	3.34 ± 0.29**	2.4
Panicum maximum	3.48 ± 0.05**	2.5
Pennisetum purpureum	3.92 ± 0.06**	2.8
Setaria viridis	2.17 ± 0.24**	1.6
Sorghum bicolor	3.50 ± 0.09**	2.5
Zea mays	3.05 ± 0.03**	2.2

For individual species, k_{cat} values are presented as means (± SE) of three independent measurements. Significant differences between rice (*Oryza sativa*) and other plants were detected by one-way ANOVA. * and ** denote significant difference at $P < 0.05$ and $P < 0.01$, respectively.

amplified by PCR using a pair of primers (RbcL-F1 GAATCTTCTACTGGTACATGGA, RbcL-R1 TCCCCTTCTAACTTACCTACTA) which were designed in the region of *RbcL* highly conserved among higher plants and sequenced.

Results and Discussion

All plants analyzed in this study showed significantly higher k_{cat} of Rubisco than that in rice and the values ranged from 1.1-to 2.8-fold relative to rice (Table 1). The k_{cat} of Rubisco in *Calamagrostis longiseta*, *Festuca ovina*, *Phleum alpinum*, *Poa arctica* and *Phleum pratense* were relatively high among C_3 plants and showed more than 1.4-fold relative to rice. Most of C_4 plants showed higher k_{cat} than C_3 plants. Among C_4 plants, *Panicum maximum*, *Pennisetum purpureum* and *Sorghum bicolor* showed more than 2.5-fold higher k_{cat} than that in rice. These findings are consistent with previous reports that C_4 plants and cold-resistant C_3 plants possess high k_{cat} or Vmax Rubisco (Seemann et al., 1984; Sage, 2002) and it is likely to be applicable to Rubisco among Poaceae (Table 1).

Table 2. Kc of Rubisco from C_3 and C_4 plants, which showed higher k_{cat} of Rubisco.

Species	Kc (μM)	Fold (relative to rice)
Oryza sativa	9.7 ± 1.05	–
C_3 alpine plants		
Festuca ovina	14.1 ± 1.63*	1.5
Phleum alpinum	21.6 ± 1.07**	2.2
C_3 cold-resistant plants		
Phleum pratense	16.4 ± 1.28**	1.7
Poa arctica	14.6 ± 0.39**	1.5
C_4 plants		
Pennisetum purpureum	37.2 ± 1.35**	3.8
Sorghum bicolor	25.6 ± 1.27**	2.6

For individual species, Kc values are presented as means (±SE) of three independent measurements. Significant differences between rice (*Oryza sativa*) and other plants were detected by one-way ANOVA. * and ** denote significant difference at $P < 0.05$ and $P < 0.01$, respectively.

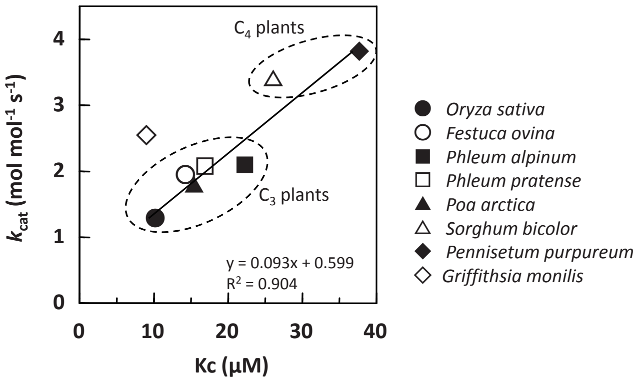

Fig. 1. Relationship between k_{cat} and Kc of Rubisco from plants used for the measurement of Kc. The data were obtained from Table 1 and 2. The regression line is indicated. The kinetic parameter of Rubisco in *Griffithsia monilis* is shown for comparison (k_{cat}=2.6 mol mol^{-1} s^{-1}, Kc=9.3 μM, Whitney et al. (2001)).

From each functional group, two species which showed high k_{cat} were selected to analyze Kc of Rubisco, namely, *F. ovina* and *P. alpinum* from C_3 alpine plants, *P. pratense* and *P. arctica* from C_3 cold-resistant plants, and *P. purpureum* and *S. bicolor* from C_4 plants (Table 2). The C_3 plants analyzed showed similar or slightly higher Kc than rice, while Kc of C_4 plants were significantly high; *P. purpureum* and *S. bicolor* showed 3.8- and 2.6-fold, respectively, higher Kc than rice. Yeoh et al. (1980; 1981) proposed that C_4 plants have a higher Kc than C_3 plants. In addition, it was suggested that there could be trade-off between k_{cat} and affinity for CO_2 in kinetics of Rubisco (von Caemmerer and Quick, 2000; Sage, 2002). This relation was clearly demonstrated by the significant positive correlation between k_{cat} and Kc in C_3 and C_4

```
                                          βC-βD loop
                                     ┌────────────────────┐
             Oryza sativa            82   GRCYHIEPVVGEDNQYIAYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Hordeum vulgare         82   GRCYHIEPVAGEDSQWICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Triticum aestivum       82   GRCYHIEPVAGEDSQWICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
C3 monocots  Festuca ovina           82   GRCYHIEPVAGEDNQWICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Phleum alpinum          82   GRCYHIEPVAGEDSQWICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Phleum pratense         82   GRCYHIEPVAGEDSQWICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Poa arctica             82   GRCYHIEPVAGEDNQWICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Pennisetum glaucum      82   GRCYHIEPVPGEADQYICYIAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Pennisetum purpureum    82   GRCYHIEPVPGEADQYICYIAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
C4 monocots  Saccharum officinarum   82   GRCYHIEPVPGDPDQYICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Sorghum bicolor         82   GRCYHIEPVPGDPDQYICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Zea mays                82   GRCYHIEPVPGDPDQYICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Arabidopsis thaliana    82   GRCYHIEPVPGEETQFIAYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALAALRLEDLRIP   141
C3 dicots    Nicotiana tabacum       82   GRCYRIERVVGEKDQYIAYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Spinacia oleracea       82   GRCYHIEPVAGEENQYICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
C4 dicots    Amaranthus tricolor     82   GRCYNIEPVAGEENQYICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
             Flaveria bidentis       82   GRCYGIEPVPGEDNQYIAYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIP   141
                                          ****.**.* *.  *.*.*.*****************************.**********
```

Fig. 2. Alignment of deduced amino acid sequence of RbcL. The partial amino acid sequences of RbcL from Gly-82 to Pro-141 (numbering based on the RbcL of *Spinacia oleracea*) are shown. The sequences of *RbcL* in *Festuca ovina, Phleum alpinum, Phleum pratense, Poa arctica, Pennisetum purpureum* and *Sorghum bicolor* were determined in this study. Asterisks and dots below the sequences represent identities and similarities, respectively. The region of βC -βD loop where RbcL interacts with Rubisco activase is indicated.

plants in Poaceae (Fig. 1). These results suggest that the higher k_{cat} seems inevitably lead to a decreased affinity for CO_2. However, Whitny et al. (2001) reported that Rubisco from the red alga *Griffithsia monilis* showed relatively high specific activity and significantly high affinity for CO_2, suggesting that the improvement of both kinetic parameters in higher plants Rubisco is theoretically possible. Among Rubisco analyzed Kc, *F. ovina*, *P. pratense* and *S. bicolor* showed relatively high k_{cat} to Kc (Fig. 1). Rubisco in these plants would be a good candidate and useful for the improvement of photosynthesis by genetic recombination in rice.

Kinetic properties of Rubisco are considered to be largely determined by *RbcL* (Andersson and Backlund, 2008). We made a comparison of the deduced amino acid sequences of RbcL among the species used in this study and representative C_3 and C_4 plants (Figs. 2, 3). Over all amino acid sequence of RbcL is well conserved among higher plants, whereas there are some natural mutations. Unfortunately, the amino acid residues that determine high k_{cat} or specificity for CO_2 have not been identified yet. As an example, one of the most variable regions including the βC -βD loop where Rubisco activase implicated to interact with RbcL (Ott et al., 2000) is shown in Fig. 2. Around this region, the differences in amino acid sequence common to the functional groups were found, for example, Ala-91 (numbering based on the RbcL of *Spinacia oleracea*) was Val in rice but Pro in C_4 plants, and Tyr-97 was Trp in C_3 alpine plant and C_3 cold -resistant plants. Also, some variations in amino acid sequence common to the functional groups were observed in the other region from Gly-82 to Arg-319 sequenced in this study. The significance or function of some amino acid residues in RbcL were investigated by site-directed mutagenesis and X-ray structural analysis (Andersson, 2008; Andersson and Backlund, 2008). However, the exact meaning of difference in amino acid sequence observed here is largely unclear. It is likely that small differences in amino acid sequence of RbcL common to the functional groups found in this study could significantly affect kinetic property of Rubisco.

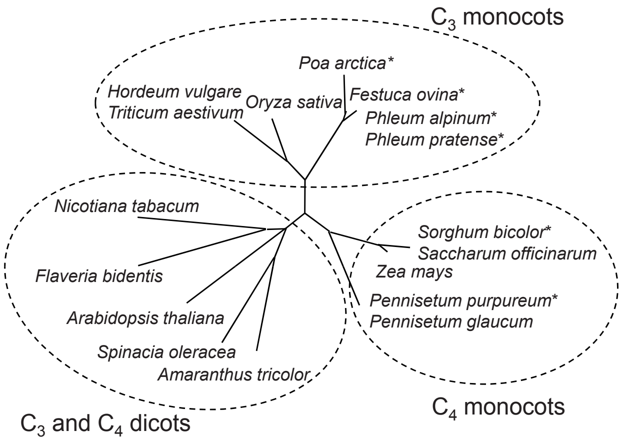

Fig. 3. The phylogenetic tree based on the partial amino acid sequence of RbcL. The phylogenetic tree was generated using the amino acid sequences of RbcL from Gly-82 to Arg-319 by the N-J method. Among dicot plants, *A. tricolor* and *F. bidentis* are C_4 plants. * means the RbcL sequences determined in this study.

Using partial amino acid sequences of RbcL, phylogenetic tree analysis was carried out to compare the sequence similarity among species (Fig. 3). Monocotyledonous and dicotyledonous plants were clustered into different groups, and monocotyledonous plants were divided into two clusters, namely, C_3 and

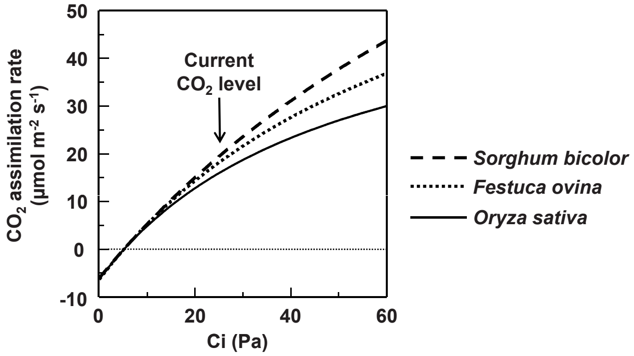

Fig. 4. Comparison of the CO_2 assimilation rate as a function of Ci modeled with kinetic parameters of Rubisco in rice (*Oryza sativa*), *Festuca ovina* and *Sorghum bicolor*. The RuBP saturated rates of CO_2 assimilation (A) at 28°C of rice and transgenic rice plants in which Rubisco were substituted by Rubisco of *F. ovina* or *S. bicolor* were calculated by the following equation (von Caemmerer and Furquhar 1981) ;

$A=(C-\Gamma^*)\cdot(k_{cat}\cdot[Rubisco])/\{C+Kc(1+O/Ko)\}-Rd$

C, the intercellular CO_2 partial pressure; O, the intercellular O_2 partial pressure; Γ^*, CO_2 compensation point in the absence of day respiration; [Rubisco], the catalytic site content of Rubisco. The k_{cat} and Kc obtained in this study were used for the calculations (*Oryza sativa* : $k_{cat}=1.39$ mol mol^{-1} s^{-1}, Kc=31 Pa ; *F. ovina* : $k_{cat}=2.05$ mol mol^{-1} s^{-1}, Kc=45 Pa; *S. bicolor* : $k_{cat}=3.50$ mol mol^{-1} s^{-1}, Kc=81.7 Pa). Ko (34.6 kPa, Makino et al. (1985)), Γ^* (4.52 Pa, Fukayama et al., unpublished data) and Rd (0.66 μmol m^{-2} s^{-1} Fukayama et al., unpublished data) were used and [Rubisco] was assumed 43.6 μmol sites m^{-2} (3.0 g Rubisco m^{-2}). Bunsen absorption coefficients were used to convert Kc and Ko values from concentration to partial pressure. The Q_{10} values of Ko (Woodrow and Berry, 1988), Γ^* and k_{cat} (von Caemmerer, 2000) were used to calculate the parameters at 28°C.

C_4 plants. Although the differences are small, RbcL of rice shares higher sequence identity with C_3 plants, *F. ovina* (96.6%) and *P. pratense* (96.2%) than that of C_4 plant, *S. bicolor* (95.4%), suggesting that RbcL of *F. ovina* and *P. pratense* would be better candidate genes to improve photosynthesis of rice. However, Rubisco of *S. bicolor* showed considerably high k_{cat} compare to the C_3 plants and this property is considered to be the most important factor for screening a better Rubisco for rice under elevated CO_2. Using the model of von Caemmerer and Farquhar (1981), the simulation of photosynthesis in rice expressing Rubisco of *F. ovina* or *S. bicolor* were demonstrated as a function of Ci (Fig. 4). In this simulation, we assumed that the values of Ko and Γ^* were constant. These parameters can vary with plant species and affect the Rubisco specificity factor. In fact, Galmes et al. (2005) reported the existence of significant variation of Rubisco specificity factor (VcKo/VoKc, where Vc, Vo and Ko are maximum carboxylation rate, maximum oxygenation rate and Michaelis constant for O_2, respectively) within higher plants. However, their simulation demonstrates that Vc and Kc are substantial determinants of photosynthetic rate. It is also apparent from the model that the effects of Ko and Γ^* on photosynthetic rate is reduced with increasing CO_2 partial pressure. These observations would support the significance of our simulation focused on the photosynthetic rate under elevated CO_2. In our estimation, the expression of *F. ovina* or *S. bicolor* Rubisco in rice could slightly enhance the photosynthetic rate at typical current CO_2 partial pressure around Ci of 25 Pa. Photosynthesis was more enhanced by the Rubisco at a higher Ci and the CO_2 assimilation rates at Ci of 50 Pa were increased 21 and 40% by Rubisco of *F. ovina* and *S. bicolor*, respectively, suggesting that these rice would have an advantage in the near future atmospheric condition over wild-type rice. Moreover, it is considered that Rubisco activity becomes excess under elevated CO_2. Makino et al. (1997) reported that the specific reduction of Rubisco content by antisence improved the nitrogen use efficiency and stimulated the photosynthesis at elevated CO_2 in rice. If rice acquired the high k_{cat} Rubisco, a lesser amount of Rubisco would be sufficient to support photosynthesis. By expressing a proper amount of high k_{cat} Rubisco in rice, it should be possible to reallocate a large amount of nitrogen to the other photosynthetic proteins and could significantly enhance photosynthesis, nitrogen use efficiency and finally, crop productivity in the future.

Acknowledgments

We would like to thank Mr. Yasuyuki Kodaka, Hakusan Alpine Plants Research Group, for supplying alpine plants, Dr. Yasuyuki Ishii, University of Miyazaki, for supplying napier grass and Dr. Hitoshi Nakagawa, National Institute of Agrobiological Sciences, for supplying sorghum and guinea grass.

References

Andersson, I. 2008. Catalysis and regulation in Rubisco. J. Exp. Bot. 59 : 1555-1568.

Andersson, I. and Backlund, A. 2008. Structure and function of Rubisco. Plant Physiol. Biochem. 46 : 275-291.

Butz, N.D. and Sharkey, T.D. 1989. Activity ratios of ribulose-1,5-bisphosphate carboxylase accurately reflect carbamylation ratios. Plant Physiol. 89 : 735-739.

Evans, J.R. 1986. The relationship between carbon-dioxide-limited photosynthetic rate and ribulose-1,5-bisphosphate-carboxylase content in two nuclear-cytoplasm substitution lines of wheat, and the coordination of ribulose-bisphosphate-carboxylation and electron-transport capacities. Planta 167 : 351-358.

Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78 : 9-19.

Evans, J.R. and Austin, R.B. 1986. The specific activity of ribulose-1,5-bisphosphate carboxylase in relation to genotype in wheat. Planta 167 : 344-350.

Galmes, J., Flexas, J., Keys, A.J., Cifre, J., Mitchell R.C., Madgwick, P.J., Haslam, R.P., Medrano, H. and Parry, M.A.J. 2005. Rubisco specificity factor tends to be larger in plant species from drier habitats and in species with persistent leaves. Plant Cell Environ. 28 : 571-579.

Kubien, D.S., von Caemmerer, S., Furbank, R.T. and Sage, R.F. 2003. C_4 photosynthesis at low temperature. A study using transgenic plants with reduced amounts of Rubisco. Plant Physiol. 132 : 1577-1585.

Li, L.R., Sisson, V.A. and Kung, S.D. 1983. Relationship between the kinetic properties and the small subunit composition of *Niconiana* ribulose-1,5-bisphosphate carboxylase. Plant Physiol. 71 : 404-408.

Makino, A., Mae, T. and Ohira K. 1983. Photosynthesis and ribulose 1,5-bisphosphate carboxylase in rice leaves. Changes in photosynthesis and enzymes involved in carbon assimilation from leaf development through senescence. Plant Physiol. 73 : 1002-1007.

Makino, A., Mae, T. and Ohira, K. 1985. Photosynthesis and ribulose-1,5-bisphosphate carboxylase/oxygenase in rice leaves from emergence through senescence. Quantitative analysis by carboxylation/oxygenation and regeneration of ribulose 1,5-bisphosphate. Planta 166 : 414-420.

Makino, A., Mae, T. and Ohira, K. 1987. Variations in the contents and kinetic properties of ribulose-1,5-bisphosphate carboxylases among rice species. Plant Cell Physiol. 28 : 799-804.

Makino, A., Sakashita, H., Hidema, J., Mae, T., Ojima, K. and Osmond, B. 1992. Distinctive responses of ribulose-1,5-bisphosphate carboxylase and carbonic anhydrase in wheat leaves to nitrogen nutrition and their possible relationships to CO_2-transfer resistance. Plant Physiol. 100 : 1737-1743.

Makino, A., Shimada, T., Takumi, S., Kaneko, K., Matsuoka, M., Shimamoto, K., Nakano, H., Miyao-Tokutomi, M., Mae, T. and Yamamoto, N. 1997. Does decrease in ribulose-1,5-bisphosphate carboxylase by antisense RbcS lead to a higher N-use efficiency of photosynthesis under conditions of saturating CO_2 and light in rice plants? Plant Physiol. 114 : 483-491.

Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8 : 4321-4326.

Ott, C.M., Smith, B.D., Portis, A.R. Jr. and Spreitzer, R.J. 2000. Activase region on chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase. Nonconservative substitution in the large subunit alters species specificity of protein interaction. J. Biol. Chem. 275 : 26241-26244.

Sage, R.F. 2002. Variation in the k_{cat} of Rubisco in C_3 and C_4 plants and some implications for photosynthetic performance at high and low temperature. J. Exp. Bot. 53 : 609-620.

Seemann, J.R., Badger, M.R. and Berry, J.A. 1984. Variations in the specific activity of ribulose-1,5-bisphosphate carboxylase between species utilizing differing photosynthetic pathways. Plant Physiol. 74 : 791-794.

von Caemmerer, S. 2000. Modeling C_3 photosynthesis. Biochemical models of leaf photosynthesis. CSIRO Publishing, Collingwood, Australia. 29-70.

von Caemmerer, S. and Farquhar, G.D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153 : 376-387.

von Caemmerer, S. and Quick W.P. 2000. Rubisco : physiology *in vivo*. In R.C. Leegood, T.D. Sharkey and S. von Caemmerer eds., Advances in Photosynthesis and Respiration, Springer, Dordrecht, Netherlands. 85-113.

Whitney, S.M., Baldet, P., Hudson, G.S. and Andrews, T.J. 2001. Form I Rubiscos from non-green algae are expressed abundantly but not assembled in tobacco chloroplasts. Plant J. 26 : 535-547.

Woodrow, I.E. and Berry, J.A. 1988. Enzymatic regulation of photosynthetic CO_2 fixation in C_3 plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39 : 533-594.

Yeoh, H.-H., Badger, M.R. and Watson, L. 1980. Variations in Km(CO_2) of ribulose-1,5-bisphosphate carboxylase among grasses. Plant Physiol. 66 : 1110-1112.

Yeoh, H.-H., Badger, M.R. and Watson, L. 1981. Variation in kinetic properties of ribulose-1,5-bisphosphate carboxylase among plants. Plant Physiol. 67 : 1151-1155.