Photosynthetic Characteristics of Antisense Transgenic Rice Expressing Reduced Levels of Rubisco Activase

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Abstract: The activation of rubulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which catalyses CO₂ fixation in photosynthesis, requires the assistance of the regulatory protein Rubisco activase. Rubisco activase promotes carbamylation of Rubisco by releasing inhibitory sugar phosphates bound to the catalytic site of Rubisco in the light. To clarify the effects of Rubisco activase contents on the photosynthesis of rice, we investigated the steady-state photosynthesis and light-induction of photosynthesis in transgenic rice plants, in which leaf Rubisco activase levels were reduced. The reduction in Rubisco activase did not affect steady-state photosynthesis under high light intensity until the Rubisco activase was about 15% of that in control plants. However, light-induction of photosynthesis, namely, increase in photosynthetic rate following a transition from a low to high light intensity, was considerably low in transgenic rice plants with 20–25% Rubisco activase, which was sufficient to support the steady-state photosynthesis. In addition, the Rubisco activase content was highly correlated with the initial rate of Rubisco activation after the increase in light intensity. These results suggest that Rubisco activase in rice leaves largely limits the light-induction of photosynthesis, but not steady-state photosynthesis.

Key words: Antisense RNA, Light-induction of photosynthesis, Rubisco activase, Steady-state photosynthesis, Transgenic rice.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase, a chloroplastic protein encoded by nuclear genes, is essential for the activation of Rubisco, which catalyzed photosynthetic CO₂ fixation. Rubisco activase promotes removal of inhibitory sugar phosphates that are bound to the catalytic site of non-carbamylated and carbamylated Rubisco in the light (Robinson and Portis, 1988, 1989a; Wang and Portis, 1992). Rubisco activase is also involved in inhibition of photosynthesis by heat stress. Depending on plant species, the heat stress induces denaturation of Rubisco activase (Grafs-Brandner and Salvucci, 2000; Salvucci et al., 2001), association of Rubisco activase with the thylakoid membrane (Rokka et al., 2001), and appearance of new Rubisco activase isoform (Sánchez et al., 1995; Law et al., 2001), and these result in decreased photosynthetic rates.

Rice has two Rubisco activase isoforms with different amino acid sequences at C termini, and their mRNAs are transcribed from a single gene by alternative splicing (To et al., 1999). The expression of genes encoding Rubisco activase (Rca) of rice is high in green organs, especially in leaf blades and leaf sheaths, but almost undetectable in roots. In the leaf blades of rice, Rca expression shows a circadian rhythm the same as observed in other plants (To et al., 1999).

Nitrogen levels rather than light intensity determine Rubisco activase contents of rice leaves (Uchida et al., 1995). During leaf aging, the Rubisco activase content showed largest changes among leaf constituents (Fukayama et al., 1996), and it is probably associated with changes in the leaf nitrogen content. It was also reported that the changes in a Rubisco activase/Rubisco ratio during leaf aging correlated well with the number of Rubisco carbamylated sites under high light intensity (Fukayama et al., 1998a) and initial rates of Rubisco activation after sudden increase of light intensity (Fukayama et al., 1998b). These findings suggested that the Rubisco activase/Rubisco ratio is one of the important factors determining rice photosynthesis during a life span of a leaf.

In order to clarify the effect of Rubisco activase on photosynthesis, transgenic plants with reduced Rubisco activase contents have been produced by the antisense RNA or RNAi technology in some plant species, tobacco (Mate et al., 1993), Arabidopsis thaliana (Eckardt et al., 1997), rice (Jin et al., 2004, 2006) and Flaveria bidentis (von Caemmerer et al., 2005). In rice, about 30% reduction in the Rubisco activase content led to 50% decrease in steady-state photosynthesis at high light in ambient air conditions.
Rubisco activase also plays an important role in determining the light-induction of photosynthesis, which is the nonsteady-state photosynthesis after a sudden increase in light intensity from dark or low light. Three phases are involved in the light-induction of photosynthesis; the regeneration of ribulose-1,5-bisphosphate (RuBP) a few seconds after the increase in light intensity, the activation of Rubisco mediated by Rubisco activase, and the changes in intercellular CO₂ concentration due to stomatal opening in the following period (Seemann et al., 1988; Kirschbaum and Pearcy, 1988; Woodrow and Mott, 1989). Usually, the rate of Rubisco activation and increase in stomatal conductance largely limit the light-induction of photosynthesis among three phases, since RuBP pool size rapidly increased just after the increase in light intensity (Seemann et al., 1988). In rice, the response of stomatal conductance is quite rapid compared with the photosynthetic rate (Fukayama et al., 1998b). In spinach leaves, the response of stomatal conductance was slower than activation of Rubisco (Woodrow and Mott, 1989). Their observations imply that the activation of Rubisco by Rubisco activase could be more important to determine the nonsteady-state photosynthesis in rice than that in other plant species. However, the effect of Rubisco activase on the light-induction of photosynthesis has not been precisely examined using *Rca antisense* transgenic rice.

To clarify the effects of Rubisco activase contents on the photosynthesis of rice, we studied the steady-state photosynthesis and light-induction of photosynthesis in transgenic rice plants with reduced levels of Rubisco activase.

Materials and Methods

1. Plant transformation and growth conditions

The cDNA encoding an open reading frame for the Rubisco activase small isoform (GenBank ID: AB034748) was isolated from rice (*O. sativa* cv. Nipponbare) by RT-PCR. Obtained cDNA was fused to the rice chlorophyll a/b binding protein (Cab) promoter in the antisense orientation and it was cloned into a binary vector pBI121Hm. For production of control plant, a binary vector excluding Rubisco activase cDNA was also constructed. The resultant plasmids were introduced into calli using *Agrobacterium*-mediated transformation (Toki, 1997). All transformants were planted in a 1 L pot containing commercial soil with 0.3 g of N, P and K, and grown in a growth cabinet under following conditions; ambient air, photosynthetic photon flux density (PPFD) of 500-700 µmol m⁻² s⁻¹, day/night (14hr/10hr) air temperature of 25°C/20°C and relative humidity of 60%. For about 60 d after germination, two T1 *Rca antisense* transgenic lines expressing Rubisco activase at 5–25% of control plants were used for experiments.

2. Steady-state photosynthetic rate

Gas exchange rates were determined with an open gas-exchange system (LI-6400, LI-COR, Lincoln, NE) equipped with a light-source (LI-6400-40, LI-COR, Lincoln, NE). The uppermost fully expanded leaves (the 9th leaves) of 5 to 8 plants per *Rca antisense* and control transgenic rice lines were used. All measurements were conducted at 21% O₂, a leaf temperature of 25°C and a vapor pressure deficit (VPD) of about 1.1 kPa. Steady-state photosynthetic rates were measured at PPFD of 250, 500, 1000 and 1500 µmol m⁻² s⁻¹ with an ambient CO₂ concentration (Cₐ) of 350 µL L⁻¹ or at a PPFD of 1500 µmol m⁻² s⁻¹ with a Cₐ of 200, 350, 500, 700 or 1000 µL L⁻¹. A leaf was placed into a chamber and incubated for 20-30 min in each condition until the photosynthetic rate reached a steady-state. Gas exchange parameters were calculated according to von Caemmerer and Farquhar (1981). After measuring photosynthetic rate, a part of the leaf inserted in the chamber was rapidly frozen in liquid N₂ and stored at −80°C until use. The rest of the leaf was dried at 80°C for 3 d for measurement of nitrogen contents.

3. Light-induction of photosynthesis

Light-induction of photosynthesis was measured with the uppermost fully expanded leaves (the 9th leaves) of 5 to 6 plants per *Rca antisense* and control transgenic rice line according to Fukayama et al. (1998b). Measurements were conducted at 350 µL L⁻¹ Cₐ, 21% O₂, a leaf temperature of 25°C and a VPD of about 1.1 kPa. A leaf placed in the chamber was illuminated for 30 min at PPFD of 1500 µmol m⁻² s⁻¹ and next PPFD was reduced to 50 µmol m⁻² s⁻¹ and further illuminated for 1hr. Then, the light intensity was returned to PPFD of 1500 µmol m⁻² s⁻¹ and gas exchange rates were recorded at intervals of 5 s for 20 min after increases in PPFD. The obtained photosynthetic rate was normalized to 250 µL L⁻¹ Cₐ by a linear regression between the photosynthetic rate at 350 µL L⁻¹ and CO₂ compensation point; the latter was fixed at 50 µL L⁻¹ in this calculation.

The relaxation time for the induction of photosynthesis after transition from low PPFD to high PPFD was calculated according to Woodrow and Mott (1989). The normalized photosynthetic rate to a Cₐ 250 µL L⁻¹ was plotted as the natural logarithm of difference between the final rate of steady-state photosynthesis and the rate at each time. The
slope of linear phase, a few minutes after the change in PPFD indicates the apparent rate constant of Rubisco activation (Woodrow et al., 1996). The apparent rate constant of photosynthetic activation was determined by a slope of linear phase that occurred from 0.5 to 5.0 min after the increase in PPFD. The relaxation time ($\tau$) was calculated as the reciprocal of apparent rate constant. This value is an indicator of time required to complete Rubisco activation. The initial rate of Rubisco activation ($V_i$) was calculated by the method of Mott et al. (1997) using Michaelis-Menten constants of rice Rubisco for CO$_2$ (256 $\mu$mol mol$^{-1}$) and O$_2$ (358 mmol mol$^{-1}$) (Makino et al., 1985; converted to mole fractions by the Bunsen coefficient).

4. Rubisco activase, Rubisco, soluble protein, chlorophyll and nitrogen contents

About 2 cm$^2$ of the frozen leaf tissues were homogenized in 2 mL of 50 mM Na-phosphate buffer (pH 7.5) containing 5 mM dithiothreitol (DTT), 0.1 mM ethylene diamine tetraacetic acid (EDTA) and 125 $\mu$L L$^{-1}$ glycerol with a chilled mortar and pestle with 5 g L$^{-1}$ polyvinylpyrrolidone (PVPP) and a small amount of acid washed quartz sand. The homogenate was centrifuged at 15,000 × g, 4ºC for 10 min and the supernatant was used for determination of Rubisco activase, Rubisco and soluble protein contents. Rubisco activase and Rubisco contents were determined by an enzyme-linked immunosorbent assay using purified Rubisco activase and Rubisco from rice leaves as standards and polyclonal antibodies against each enzyme (Masumoto et al., 2004). Soluble protein contents were determined according to Bradford (1976) using bovine serum albumin as a standard. For measurements of chlorophyll contents, leaves were homogenized with 80% acetone and quantified as described by Porra et al. (1989). Nitrogen content was determined by the indophenol colorimetric method after Kjeldahl digestion of dried leaf samples following incubation at 80ºC for 3 days.

5. Statistical analysis

Statistical analysis was performed with the Tukey-Kramer multiple comparison test using R software (version 2.10.1).

### Table 1. Contents of nitrogen, soluble protein, Rubisco and chlorophyll, and a ratio of chlorophyll a/b in leaves of control and Rea antisense plants.

|                | Nitrogen (g m$^{-2}$) | Soluble protein (g m$^{-2}$) | Rubisco (g m$^{-2}$) | Chlorophyll (mmol m$^{-2}$) | Chlorophyll a/b |
|----------------|-----------------------|-------------------------------|----------------------|-----------------------------|-----------------|
| Control        | 1.63 ± 0.22 a         | 6.01 ± 0.67 a                 | 2.96 ± 0.31 a        | 0.51 ± 0.05 a               | 2.90 ± 0.18 a   |
| Rea antisense (AM) | 1.61 ± 0.34 a      | 6.30 ± 1.13 a                 | 3.43 ± 0.37 a        | 0.48 ± 0.07 a               | 2.94 ± 0.22 a   |
| Rea antisense (AS) | 1.55 ± 0.10 a       | 6.18 ± 0.76 a                 | 3.09 ± 0.34 a        | 0.50 ± 0.02 a               | 3.07 ± 0.06 a   |

Data are presented as average ± SD (n = 5 – 8). Means followed by the same letter do not differ significantly at 5% level.
reduction below this level considerably decreased the photosynthetic rate (Fig. 1). About 90% reduction in Rubisco activase contents decreased the photosynthetic rate to 50% of the control (Fig. 1).

For further analysis, *Rca* antisense transgenic rice plants were divided into two groups: AM and AS plants (*Rca* antisense transgenic rice with moderately and severely reduced Rubisco activase, respectively). AM plants expresses Rubisco activase reduced to 20−25% of that in control plants with unchanged steady-state photosynthesis under high PPFD and 350 µL L\(^{-1}\) CO\(_2\) conditions (Fig. 1). AS represents Rubisco activase reduced to 5−15% of the control showing reduced photosynthetic rates compared with those of control plants (Fig. 1).

We then examined the effects of reduced Rubisco activase level on nitrogen, soluble protein, Rubisco and chlorophyll contents of leaves. Soluble protein and Rubisco contents tended to be higher in *Rca* antisense plants, although all components measured in *Rca* antisense plants were not significantly different from those in the control (Table 1).

2. **Steady-state photosynthetic rate**

The reduction in Rubisco activase significantly affected the photosynthetic rates at a higher PPFD. In AS plants, the photosynthetic rates above a PPFD of 500 µmol m\(^{-2}\) s\(^{-1}\) were significantly reduced to 70% of the control plants, while they remained 90% of the control at PPFD of 250 µmol m\(^{-2}\) s\(^{-1}\) (Fig. 2A). The photosynthetic rate in AM plants were similar to that in the control plants at all four PPFDs examined (Fig. 2A). The photosynthetic rate in AS plants was lower than that in the control plants, but the difference became smaller with increasing intercellular CO\(_2\) concentration (C\(_i\)) (Fig. 3A). At a C\(_i\) of about 900 µL L\(^{-1}\), the photosynthetic rate in AS plants was similar to that in the control (Fig. 3A). At a relatively high C\(_i\), AM plants tended to exhibit higher photosynthetic rates than the control plants (Fig. 3A). Both AM and AS plants had a stomatal conductance similar to that in control plants under various PPFD (Fig. 2B) and C\(_i\) (Fig. 3B). The ratio of C\(_i\) to ambient CO\(_2\) concentration (C\(_i)/C_o\) ratio) in AS plants was higher than that in control plants at four PPFDs (Fig. 2C) and at 200 to 500 µL L\(^{-1}\) C\(_o\) (Fig. 3C), while the C\(_i)/C_o\) ratio in AM plants was similar to that in the control (Figs. 2C, 3C).

3. **Light-induction of photosynthesis**

We then examined the effects of reduction in Rubisco activase on light-induction of photosynthesis after a sudden increase in PPFD under the ambient air condition. PPFD of 50 µmol m\(^{-2}\) s\(^{-1}\) was used as the initial low light, since Rubisco activase greatly limited the light-induction of photosynthesis following a transition from PPFD below 200 µmol m\(^{-2}\) s\(^{-1}\) to high PPFD (Fukayama et al., 1998b). The

![Fig. 2. Dependence of steady-state photosynthesis, stomatal conductance and a ratio of intercellular (C\(_i\)) to ambient CO\(_2\) concentrations (C\(_o\)) on light intensity in control and *Rca* antisense plants. AM (open square) and AS (open triangle) of *Rca* antisense plants contained Rubisco activase reduced to 20−25% and 5−15% of the control (closed circle), respectively. Measurements were made under 350 µL L\(^{-1}\) C\(_o\), 21% O\(_2\), leaf temperature of 25°C and VPD of about 1.1 kPa. *** and ** indicate significant differences between control and *Rca* antisense plants at 0.1 and 1% levels, respectively. Data are given as average±SD (n=5−8).]
Fig. 3. Dependence of steady-state photosynthesis and stomatal conductance on intercellular CO₂ concentration (Cᵢ), and the ratio of Cᵢ to ambient CO₂ concentrations (Cᵢ/Cₐ) on Cᵢ in control and Rca antisense plants. AM (open square) and AS (open triangle) of Rca antisense plants contained Rubisco activase reduced to 20–25% and 5–15% of the control (closed circle), respectively. Measurements were made under PPFD of 1500 µmol m⁻² s⁻¹, 21% O₂, leaf temperature of 25ºC and VPD of about 1.1 kPa. ***, ** and * indicate significant differences between control and Rca antisense plants at 0.1, 1 and 5% levels, respectively. Data are given as average±SD (n=5–8).

Fig. 4. Representative patterns of time courses for photosynthetic rate, stomatal conductance and intercellular CO₂ concentration (Cᵢ) to a sudden increase in PPFD from 50 to 1500 µmol m⁻² s⁻¹ in control and Rca antisense plants. AM (open square) and AS (open triangle) of Rca antisense plants contained Rubisco activase reduced to 20–25% and 5–15% of the control plants (closed circle), respectively. PPFD was increased from 50 to 1500 µmol m⁻² s⁻¹ at time zero. Measurements were made under 350 µL L⁻¹ CO₂, 21% O₂, leaf temperature of 25ºC and VPD of about 1.1 kPa.
increase in photosynthetic rate after light transition was clearly slower in \textit{Rca} antisense plants than that in the control plants (Fig. 4A). Even AM plants that exhibited similar levels of steady-state photosynthetic rates showed slower activation of photosynthesis (Fig. 4A). The photosynthetic rate in AM plants gradually increased for 10 min after the transition to high PPFD, after which they reached a steady state, while that in the control reached a steady state in a shorter time of about 5 min (Fig. 4A). The AS plants showed much slower activation of photosynthesis compared with AM plants and did not reach a steady state within 15 min (Fig. 4A). There was no difference in the stomatal conductance after the light transition between \textit{Rca} antisense and control plants (Fig. 4B). The stomatal conductance rapidly increased during the first 2 min, and then reached a steady state earlier than did the photosynthetic rate (Fig. 4B). The $C_i$ remained almost constant after the light transition, although $C_i$ in \textit{Rca} antisense plants tended to be slightly higher than that in control plants (Fig. 4C).

Using the data of gas exchange measurements, we calculated the relaxation time ($\tau$) for increased photosynthetic rate after the transition from low PPFD to high PPFD. The photosynthetic rates were normalized to $C_i$ of 250 $\mu$L L\textsuperscript{-1} for calculation of $\tau$, as changes in $C_i$ might influence the photosynthetic rate. The $\tau$ in \textit{Rca} antisense was essentially higher than that in control plants (Fig. 5A); average $\tau$ in AM, AS and the control plants were 7.76, 5.54 and 2.78 min, respectively. The lower value of $\tau$ in AS than in AM is probably due to under estimation of the final steady-state photosynthetic rate in AS plants, which did not reach a steady state within our measurement time because of the extremely slower activation of photosynthesis (Fig. 4A). The gas exchange data revealed a high positive correlation between Rubisco activase content and initial rate of Rubisco activation in AM with moderately reduced Rubisco activase as well as in the other plants (Fig. 5B).

**Discussion**

Using the transgenic plants with reduced Rubisco activase levels, we examined the correlation of the Rubisco activase level with steady-state photosynthesis, and the light-induction of photosynthesis after the transition from a low to high light intensity.

The Rubisco activase contents of rice leaves were found to be saturated in the steady-state photosynthesis. Severe reduction of Rubisco activase to less than 15% of that in the control plants was required to decrease the photosynthetic rates (Fig. 1). In \textit{Rca} antisense transgenic rice with Rubisco activase reduced to 20 – 25%, the photosynthetic rates was similar to those of control plants (AM in Figs. 2A, 3A), but Rubisco activase contents below this level led to significant decrease in the photosynthetic rates, even at relatively high $C_i$ of about 400 and 600 $\mu$L L\textsuperscript{-1} (AS in Figs. 2A, 3A), where electron transport rate or Pi regeneration capacity limited the photosynthetic rate (Sharkey, 1985). High $C_i/C_a$ ratio of AS plants (Figs. 2C, 3C) indicated that the decreased photosynthetic rate in AS plants was not ascribed to low $C_i$. These results are well consistent with those in other plant species, tobacco (Mate et al., 1993) and \textit{Arabidopsis} (Eckardt et al., 1997), suggesting that these plants contained an excess amount of Rubisco activase for steady-state photosynthesis.

Contrary to our results, Jin et al. (2004, 2006) reported that in the rice cultivar ZhongHua 11, moderate reduction of Rubisco activase to 70% of the control considerably decreased the photosynthetic rates in \textit{Rca} antisense transgenic plants, suggesting that Rubisco activase in leaves could be a more important determinant of photosynthesis.
in rice compared with other plant species. In their report, leaf Rubisco activase and Rubisco content of ZhongHua 11 were lower than those of Nipponbare in this study, though this might be due to the difference in growth condition or detection method. It is possible that the photosynthetic rate in ZhongHua 11 is limited more severely by Rubisco activity than in Nipponbare, thus causing a greater response to the reduction in Rubisco activase. The Rubisco level was markedly increased in Rea antisense transgenic rice of ZhongHua 11, which is considered as a compensational effect in Rea antisense plants (Mate et al., 1993; Eckardt et al., 1997). This may reflect the larger effects of Rubisco activase reduction on photosynthesis in this cultivar. Although large variations in Rubisco activase levels have been reported in wheat cultivars (Ristic et al., 2009), variations in Rubisco activase levels, specific activity, and thermal stability among rice cultivars have not been investigated and remain to be determined.

In this study, transgenic rice plants with moderate reduction of Rubisco activase tended to exhibit higher photosynthetic rates at high Ci than control plants (AM in Fig. 3A). This was not attributed to an increase in Rubisco content, because over-expression of Rubisco in rice leaves did not enhance the photosynthesis at various PPFD and CO2 concentrations (Suzuki et al., 2007). Under high CO2 and high PPFD conditions, the electron transport, which supplies adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) into the chloroplast, or inorganic phosphate (Pi) regeneration capacity, which recycles adenosine diphosphate (ADP) and Pi to produce ATP, should limit the photosynthesis. Changes in the balance between ATP and ADP levels in the chloroplast might increase photosynthetic rates in the Rea antisense rice under a high CO2 condition. In addition to the Rubisco activation activity, Rubisco activase has ATPase activity, and the ATPase activity is inhibited by ADP (Robinson and Portis, 1989b). The ATP/ADP ratio generally decreases under a higher CO2 condition by an increase in ATP consumption (Gardstrom and Wigge, 1988), and this could down-regulate the activation state of Rubisco under elevated CO2 conditions (Crafts-Brandner and Salvucci, 2000). Rubisco as well as Rubisco activase levels are considered to become excessive for photosynthesis under high CO2 condition. In Rea antisense rice, the ATP/ADP ratio in the chloroplast might be relatively high by reduced consumption of ATP by Rubisco activase. This enabled the electron transport rates in Rea antisense rice to maintain high at higher CO2.

Rubisco activase was found to be more important for light-induction of photosynthesis than in steady-state photosynthesis in rice. The photosynthetic rates after light-induction increased slowly in Rea antisense transgenic rice with sufficient levels of Rubisco activase for the steady-state photosynthesis (AM in Fig. 4A). The initial rate of Rubisco activation estimated from gas exchange measurements highly correlated with Rubisco activase contents (Fig. 5B). This correlation was also observed during leaf aging in rice (Fukayama et al., 1998b). These results indicate that the Rubisco activase plays an essential role in light-activation of Rubisco, and limits the light-induction of photosynthesis throughout a lifetime of rice.

Woodrow and Mott (1989) showed that activation of Rubisco mainly limits light-induction of photosynthesis in spinach leaves because it exhibits the slowest relaxing process, with the exception of stomatal opening. Rapid increase in stomatal conductance and stability of Ci were observed in both Rea antisense rice and control plants (Figs. 4B, 4C), indicating that the stomatal opening cannot be a limiting factor for the light-induction of photosynthesis under our experimental conditions. The mechanisms of light-activation of Rubisco varies with the plant species. Some plants such as Phaseolus vulgaris accumulate an inhibitor 2-carboxy-D-arabinitol 1-phosphate (CA1P) which tightly binds to the carbamylated forms of Rubisco during the night (Moore et al., 1991). On the other hand, most plants do not accumulate CA1P and in these plants, RuBP bound to the non-carbamylated forms of Rubisco under low light conditions (Cardon and Mott, 1989). Rubisco activase can mediate the release of both CA1P and RuBP from Rubisco. Although the levels of CA1P in rice leaves have not been directly measured, rice leaves probably contain CA1P because Rubisco activation remains relatively high in the dark or at a low PPFD in the vegetative stage (Yamauchi et al., 2001), as observed in CA1P accumulating plants. However, low Rubisco activation in the dark was also reported in the leaves of a different rice cultivar at the heading stage (Chen et al., 2010), and this suggested that CA1P levels may vary with the growth stage and/or cultivar. In P. vulgaris and Beta vulgaris containing high CA1P, the Rubisco activation after a sudden increase in the light intensity was faster than in S. oleracea containing low CA1P (Kobza and Seemann, 1988). These observations suggest that the release of RuBP from non-carbamylated Rubisco by Rubisco activase is an essential limiting step in light-induction of photosynthesis in rice.

Our experiments with Rea antisense rice showed that Rubisco activase in rice leaves was an important determinant in nonsteady-state photosynthesis, while its content was sufficient for the steady-state photosynthesis. At higher temperature, the steady-state photosynthesis in rice can be influenced by Rubisco activase. Yamori and von Cammerer (2009) reported that steady-state photosynthetic rate at higher temperature decreased in transgenic tobacco containing reduced Rubisco activase, which exhibited the same levels of photosynthesis as the wild type at growth temperature. Since Rubisco activase limitation on photosynthesis may vary with the rice cultivar, it can be physiologically meaningful and also useful for
improvement of photosynthetic efficiency in rice to analyze the difference in the potential of light-induction and heat tolerance of photosynthesis induced by Rubisco activase among various cultivars and wild relatives.

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