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Characteristics of photosynthesis in rice plants transformed with an antisense Rubisco activase gene[*]

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Abstract: Transgenic rice plants with an antisense gene inserted via *Agrobacterium tumefaciens* were used to explore the impact of the reduction of Rubisco activase (RCA) on Rubisco and photosynthesis. In this study, transformants containing 15% to 35% wild type Rubisco activase were selected, which could survive in ambient CO_2 concentration but grew slowly compared with wild type controls. Gas exchange measurements indicated that the rate of photosynthesis decreased significantly, while stomatal conductance and transpiration rate did not change; and that the intercellular CO_2 concentration even increased. Rubisco determination showed that these plants had approximately twice as much Rubisco as the wild types, although they showed 70% lower rate of photosynthesis, which was likely an acclimation response to the reduction in Rubsico activase and/or the reduction in carbamylation.

Key words: Antisense *rca* rice plants, Rubisco, Rubisco activase, Photosynthesis
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INTRODUCTION

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a key enzyme that initiates both photosynthetic and photorespiratory carbon metabolism. However, Rubisco has to be activated and carbamylated to become catalytically competent. Rubisco activase (RCA) can alter the activity of Rubisco by facilitating the dissociation of tightly bound sugar phosphates from Rubisco in a process that requires ATP hydrolysis. RCA, a chloroplast protein encoded by a nuclear gene, has become of interest to many agricultural investigators because the enzyme may be a means to improve the net rate of photosynthesis (Spreitzer and Salvucci, 2002).

With the rapid development of biotechnology, increasing numbers of RCA genes (*rca*) have been cloned from different plants. Antisense RNA techniques provide ideal means to examine the function of specific proteins. The antisense tobacco plants (Mate *et al*., 1993; 1996; Hammond *et al*., 1998) and *Arabidopsis* (Eckardt *et al*., 1997; Zhang *et al*., 2002) with reduced RCA levels were produced in previous studies by this technique, and the role of RCA was investigated in these plants. But the precise mechanism of RCA remained unclear, especially in rice, a monocotyledonous model plant. To explore the role of RCA in rice plants, we firstly produced transgenic antisense *rca* rice. In this work, these plants were used to further examine the relationship between Rubisco, RCA and photosynthesis.

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MATERIALS AND METHODS

Plants used in this experiment were the T_1 progeny of ZhongHua 11 (*Oryza sativa* L. ssp *japonica*) transformed with an antisense *rca* gene by the *Agrobacterium tumefaciens* system (Jing *et al*., 2004). The seedlings of the T_1 transgenic rice were selected by hygromycin and transplanted as described in our previous report (Jiang *et al*., 2001).

Gas exchange was determined with a portable photosynthesis system (LiCor-6400; LiCor Inc. Lincoln, Nebraska, USA) and a LED light source, 6400-02. This experiment was conducted at light intensity of 1200 μmol/m^2·s, leaf temperature of 28 °C, and constant CO_2 of 380±5 μmol CO_2/mol in the sample chamber provided with buffer volume. Measurements for antisense *rca* rice and wild type plants were made on the second youngest, fully expanded leaves from stem top of rice plants at 7th week after being transplanted.

The contents of Rubisco and RCA were measured by single radial immunodiffusion method as described by Wang *et al*.(2003). The soluble protein content was determined with the dye-binding method introduced by Bradford (1976) using bovine serum albumin as a standard.

RESULT AND DISCUSSSION

The antisense *rca* rice plants hardly grew under ambient CO_2 concentration, as more than two-thirds of them perished. This was consistent with previous findings using tobacco plants (Mate *et al*., 1993; 1996; Hammond *et al*., 1998) and *Arabidopsis* (Eckardt *et al*., 1997; Zhang *et al*., 2002). In this study, antisense rice plants which could survive in natural conditions were selected. The amounts of RCA of these plants ranged from 15% to 35% of the wild type plants. The mean contents of RCA in these plants are shown in Table 1. Under the condition of light saturation, the photosynthetic rate of the selected plants was reduced by about 70%, as compared to the wild type plants (Fig.1). There are two main factors that can cause a decrease in photosynthetic rate. The first is a stomatal factor that depends on the number of stomatal pores, stomatal location and dimensions (Quick *et al*., 1992). The other is a non-stomatal factor which primarily depends on the activity of intrinsic enzymes, photosynthetic apparatus and their regulation mechanism (Lal *et al*., 1996). To further explore the reasons for the reduction in photosynthesis of these plants, the characteristics of gas exchange and the amounts of Rubisco and soluble protein were examined. In our case, the intercellular CO_2 concentrations (Ci) of the antisense *rca* rice were about 50%, higher than that of controls in spite of their lower rate of photosynthesis, while there were no changes in stomatal conductance (g_s) and transpiration rate (E) between the antisense and wild type plants (data not shown), which indicated that the reductions of photosynthetic rate of the selected antisense plants were not due to stomatal factors.

Immunodiffusion analysis showed that the RCA levels of the selected transformants were reduced dramatically. However, the antisense *rca* plants had more soluble protein in their leaves, and had, on average, approximately twice as much Rubisco as control plants (Table 1). Therefore, there was a greater ratio of Rubisco to soluble pro-

Table 1 Rubisco, RCA and soluble protein contents in antisense *rca* rice plants and wild types[*]

Cultivars	Rubisco (g/m^2)	RCA (mg/m^2)	Soluble protein (g/m^2)
Antisense *rca*	3.22±0.61	4.50±1.61	3.35±0.26
Wild types	1.68±0.20	20.14±0.60	5.32±0.82

[*] Values are the mean±*SE* (*n*=6)

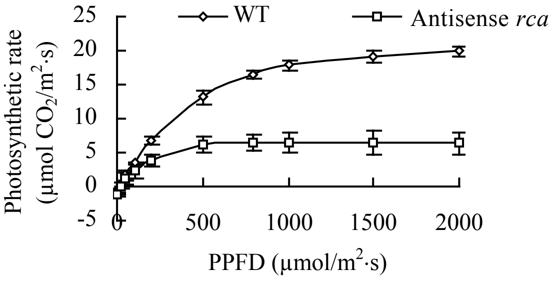

Fig.1 Light response curves of photosynthesis of antisense *rca* rice plants and wild types

tein in the antisense plants than the control plants. Although the content increased, the photosynthetic rate was still reduced remarkably because the levels of carbamylation of Rubisco may be strongly reduced as previously demonstrated in tobacco (Hammond *et al.*, 1998). The physiological role of these changes is not known, but it is tempting to speculate that they function in the acclimation of photosynthesis to the decrease in Rubisco activase, perhaps by compensating the reduction in carbamylation as a mechanism of long-term survival. We believe that the antisense plants will be useful for further examination of the regulation of the interaction between Rubisco and RCA.

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