S17-012

Effects of overproduction of the maize C_4-specific phospho*enol*pyruvate carboxylase on photosynthesis and respiration

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Keywords: C_4 photosynthesis, maize, phosphoenolpyruvate carboxylase, transgenic rice

Introduction

Phospho*enol*pyruvate carboxylase (PEPC) catalyzes the initial CO_2 fixation of photosynthesis in C_4 and CAM plants. This enzyme is also present in the leaves of C_3 plants, though its activity is low, and plays a variety of roles, e.g. an anaplerotic function replenishing the TCA cycle with intermediates, a pH stat and stomata opening. It has long been anticipated that overproduction of PEPC in the C_3 leaves would improve the efficiency of photosynthetic CO_2 assimilation. Several studies have previously reported the effects of overproduction of PEPC on photosynthesis and respiration in transgenic C_3 plants, namely, reduction of O_2 inhibition of CO_2 assimilation (Ku et al. 1999), an increase in the quantum efficiency of CO_2 assimilation at supra-optimal temperatures (Kogami et al. 1994), stimulation of respiration both in the light and in darkness after illumination (Gehlen et al.1996, Häusler et al. 1999), and enhancement of stomata opening (Ku et al. 2000). However, the results are controversial and any definitive conclusions have not been reached yet. In this study, we reexamined the effects of overproduction of PEPC using transgenic rice plants overproducing the maize C_4-specific PEPC.

Materials and Methods

Plant growth conditions

Rice plants were planted in soil and grown in a growth chamber at 25°C day/20°C night cycle with a day period for 14 h under illumination at photosynthetically active photon flux density (PPFD) of 500 μmol m^{-2} s^{-1}. Transgenic rice plants overproducing PEPC were produced by introduction of the intact maize C_4-specific PEPC gene (Ku et al. 1999). When indicated, two different homozygous lines PE84 and PE2 were used. Their activities of PEPC in the leaves, measured in the presence of glucose-6-phosphate, were 20- and 50- fold, respectively, over that of non-transgenic rice.

Gas-exchange measurements

Gas exchange was measured with an open gas exchange system (LI-6400, Li-Cor, Nebraska, USA) as described by Tsuchida et al. (2001). Unless stated otherwise, CO_2 assimilation was measured at a leaf temperature of 25°C, 360 μL/L CO_2, 21% O_2 and PPFD of 1500 μmol m^{-2} s^{-1}. The extent of O_2 inhibition of CO_2 assimilation was calculated as (rate at 2% O_2 – rate at 21% O_2)/rate at 2% O_2. Effects of temperature on CO_2 assimilation were investigated using a

single leaf by increasing the leaf temperature stepwise. Quantum yield was determined as the initial slope of the light response curve of the CO_2 assimilation rate. To measure Rn (the respiration rate in darkness) and LEDR (light enhanced dark respiration), a leaf was initially illuminated with the measuring light at PPFD of 500 μmol m^{-2} s^{-1} for 30 min, and then the light was turned off. Rn was determined as the steady-state rate of CO_2 release 20-30 min

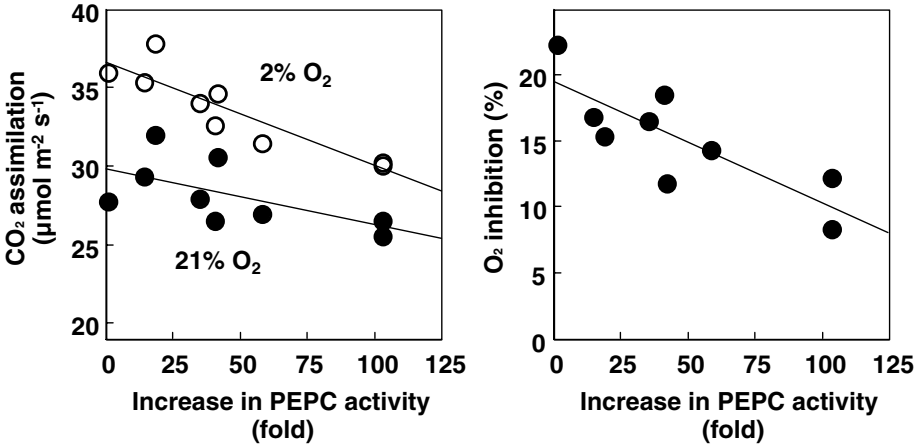

Fig. 1. The CO_2 assimilation rate (A) and O_2 inhibition of CO_2 assimilation (B) as a function of PEPC activity in the primary transgenic rice plants introduced with the intact maize PEPC gene.

after transfer to darkness. LEDR was determined as a difference between Rn and CO_2 release 3-4 min after transfer to darkness. Rd (the respiration rate under illumination independent of photorespiration) and Γ* (the CO_2 compensation point independent of respiration) were determined by the method of Brooks and Farquhar (1985).

Results and Discussion

Photosynthesis and respiration at temperatures optimal for plant growth

Transgenic rice plants overproducing the maize PEPC showed the CO_2 assimilation rate comparable to or slightly lower than that of non-transgenic rice at 25°C under ambient air conditions (21% O_2). We have previously reported that the O_2 inhibition of CO_2 assimilation was mitigated by overproduction of the maize PEPC in transgenic rice plants, and proposed that the maize PEPC participated in CO_2 fixation for photosynthesis (Ku et al. 1999). We found later that this was not the case. As shown in Fig. 1, the CO_2 assimilation rate decreased with increasing PEPC activity in both 21 and 2% O_2, with the slope of the regression line being steeper in 2% O_2 than in 21% O_2. Thus, the observed reduction of O_2 inhibition resulted from increased inhibition of CO_2 assimilation by overproduction of PEPC in 2% O_2. In addition, labeling experiments with $^{14}CO_2$ under illumination indicated that the initial CO_2 fixation product in the transformant PE2 was exclusively the C_3 compound 3-phosphoglycerate (Fukayama et al. 2000). These observations indicate that the maize PEPC does not contribute to CO_2 fixation for photosynthesis in transgenic rice.

It was reported using transgenic potato plants that overproduction of PEPC of *Corynebacterium glutamicum* decreased Γ* and increased Rd, an indication of decreased photorespiration and increased respiration in the light, respectively (Häusler et al. 1999). Although not always, we also observed an increase in Rd and a marginal decrease in Γ* in our transgenic rice plants (Table 1). Since respiration under illumination is enhanced at lower O_2 concentrations, probably due to suppression of photorespiration that can inactivate the pyruvate dehydrogenase complex, a key enzyme of the TCA cycle (Atkins et al. 1998), it is likely that the increase in Rd by overproduction of PEPC could be enlarged in 2% O_2 to

reduce the apparent rate of CO_2 assimilation to a larger extent. This would be a reason for the increased inhibition of CO_2 assimilation in 2% O_2 in transgenic rice (Fig.1). It has also been reported that overproduction of the *Corynebacterium* PEPC enhanced dark respiration after pre-illumination (Gehlen et al.1996). We did not observe any differences in either Rn after pre-illumination or LEDR between transgenic and non-transgenic rice (Table 1).

Table 1. Various charactristics of photosynthesis and respiration of transgenic rice plants overproducing the maize PEPC

		CO_2 assimilation (μmol m^{-2} s^{-1})			Apparent O_2 inhibition (%)			Quantum yield (mmol mol^{-1})		Rn	LEDR	Rd	Γ*
										(μmol m^{-2} s^{-1})			μL L^{-1}
	[O_2]	25 °C	32 °C	40 °C	25 °C	32 °C	40 °C	25 °C	35 °C				
Rice	21%	27.1 (100)	27.9 (103)	24.0 (89)	30.8 (100)	38.4 (125)	35.8 (116)	62.2 (100)	54.4 (87)	0.851	1.06	0.551	42.4
	2%	34.4 (100)	41.7 (121)	35.4 (103)									
PE 2	21%	24.2 (100)	25.1 (104)	20.4 (84)	29.0 (100)	35.0 (121)	29.2 (101)	61.8 (100)	55.4 (90)	0.779	0.97	0.662	41.2
	2%	31.4 (100)	36.1 (115)	27.6 (88)									
PE84									51.5	0.878	1.06	0.663	40.1

The numbers in parentheses indicate the percentages of the values when those at 25 °C were taken as 100%.

Photosynthesis at supra-optimal temperatures

It was reported in transgenic tobacco overproducing the maize PEPC that overproduction of PEPC increased the quantum efficiency of CO_2 assimilation at supra-optimal temperatures (Kogami et al. 1994). We did not observe such an effect in our transgenic rice (Table 1). In addition, the temperature dependence of the CO_2 assimilation rate did not differ between transgenic and non-transgenic rice under ambient air conditions (Table 1). Interestingly, in 2% O_2, the CO_2 assimilation rate at 40°C of the transformant was significantly lower than that of non-transformant, and consequently, the difference in apparent O_2 inhibition was enlarged. This result might also be ascribable to the enhanced respiration under illumination by the maize PEPC.

Stomatal conductance

Ku et al. (2000) reported that overproduction of the maize PEPC increased the stomatal conductance under steady-state conditions in transgenic rice. However, we could not detect any differences in this parameter between transgenic and non-transgenic rice: a plot of the stomatal conductance against the vapor pressure deficit at the leaf surface gave identical lines (data not shown). In contrast, the stomatal conductance under nonsteady-state conditions could differ between transgenic and non-transgenic rice (Fig. 2), as previously reported in transgenic potato overproducing the *Corynebacterium* PEPC (Gehlen et al. 1996). When leaves were transferred from low to high light intensity, the CO_2 assimilation rate and the stomatal conductance increased in a few minutes to reach new steady-state levels. Once the steady-state level was attained, the stomatal conductance tended to decrease faster in the transformant than in the non-transformant (Fig. 2). After prolonged illumination for 1-2 h, however, the final steady-state levels were comparable. It is still obscure if this phenomenon is ascribable to overproduction of PEPC in guard cells.

As described above, overproduction of the maize PEPC in transgenic rice does not significantly affect photosynthesis and respiration. Some changes we detected were stimulation of respiration under illumination and marginal suppression of photorespiration (Table 1). The extents of these effects, however, were much smaller than those previously demonstrated in transgenic potato overproducing the *Corynebacterium* PEPC (Gehlen et al. 1996, Häusler et al. 1999). Such differences might be ascribable to low *in vivo* activities of PEPC in our transgenic rice. We found that the maize PEPC in transgenic rice underwent the activity regulation through reversible phosphorylation and that it remained in the dephosphorylated less active form under illumination (Fukayama et al. 2000). In contrast, the *Corynebacterium* PEPC does not undergo such a regulation so that its activity is not downregulated even in the cytosol of the mesophyll cells of C_3 plants, in which

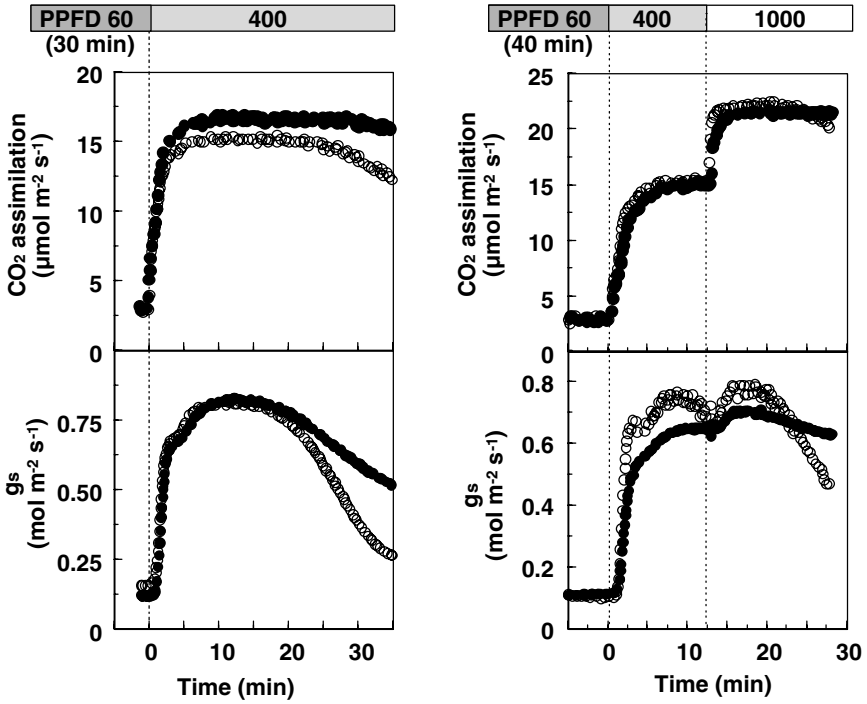

Fig. 2. The response of CO_2 assimilation rate and the stomatal conductance (gs) to an increase in light intensity. (Open symbols) non-transgenic rice; (closed symbols) PE2.

concentrations of inhibitors of PEPC are high. In accordance with the enhancement of respiration under illumination, we observed some indications of enhanced nitrogen assimilation in transgenic rice, namely, increases in the levels of aspartic and glutamic acids in the leaves and enhanced growth under nitrogen limiting conditions (data not shown).

Acknowledgements

The authors are grateful to Drs. M.D. Hatch and R.T. Furbank (CSIRO, Australia) for their help with $^{14}CO_2$ labeling experiments.

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