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Characteristics of carbon assimilation and tolerance to photo-oxidation in transgenic rice expressing C_4 photosynthesis enzymes

D-M Jiao[1], X-Q Huang [1], X Li [1], W Chi[1], T-Y Kuang[2], Q-D Zhang[2], MSB Ku[3]

[1]*Institute of Agrobiological Genetics and Physiology, Jiangsu Academy of Agricultural Sciences, Nangjing 210014, China; e-mail: photosyn@public1.ptt.js.cn.*

[2]*Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China.*

[3]*School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA.*

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Introduction

More than 95% of the terrestrial plants, including many agronomically important crop species such as rice, assimilate carbon through the C_3 pathway of photosynthesis. Under current atmospheric conditions, O_2 reduces the photosynthetic capacity in C_3 plants by as much as 30-40% due to photorespiration. Under high light, high temperature, and drought conditions, which reduce CO_2 concentration in the leaf, the photosynthetic capacity of C_3 plants would decline further (Ku et al. 1999). In plants that utilize the C_4 photosynthetic pathway, atmospheric CO_2 is initially fixed by PC in the mesophyll cells, which has a high affinity for the substrate, bicarbonate, and is not inhibited by O_2. In addition, the C_4 pathway serves as a "CO_2 pump" to concentrate CO_2 in the leaf and suppress photorespiration. Therefore, photosynthesis by C_4 plants is not limited by atmospheric levels of CO_2 and exhibits a higher photosynthetic efficiency, especially under high light, high temperature and drought conditions. It was also discovered that enzymes involved in C_4 photosynthesis play important roles in plant defense responses to biotic and abiotic stress (Ku et al. 2000). Thus, over-expression of C_4 photosynthesis enzymes in C_3 plants may improve their photosynthetic capacity and tolerance to stress.

Over the past few decades, attempts have been made to incorporate C_4 traits into C_3 crops. Some notable progress has been made from crosses made between closely related C_3 and C_4 plants using conventional hybridization approach (Jiao et al.1991; Brown et al. 1993). Although the results indicate that C_4 photosynthetic traits can be expressed in plants using this approach, but there are no closely related C_3 and C_4 crops can be used in breeding program. With the rapid development of transgenic technology in recent years, several maize genes encoding C_4 photosynthesis enzymes, such as PC, PK, and ME, have been introduced into the C_3 crops such as rice (Ku et al. 1999). PC transgenic rice expressed high levels of the maize enzyme and had high photosynthetic rates (Ku et al. 1999). Most importantly, the plants exhibited a higher yield potential (Ku et al. 2000), which demonstrates the broad prospects for improving crop photosynthetic productivity via genetic engineering. However, the photosynthetic characteristics of the transgenic rice have not been systematically examined. In this paper, we report on the characteristics of CO_2 assimilation of photosynthesis and tolerance to photo-oxidation of transgenic rice expressing the maize C_4 photosynthetic enzymes (PC, PK, ME) to provide experimental evidences for the basis of the enhanced photosynthetic capacity and yield potential in these plants to open up a new approach to physiological breeding.

Materials and Methods

The seeds of the 7th stable generation of PC and the 3rd generation of PK, ME and PC+PK transgenic rice were germinated and grown in pots in a naturally illuminated net house at Jiangsu Academy of Agriculture Sciences during May-August in 1999 and 2000. There were five hills per pot and one seeding for each hill. The activity and protein amount of the C_4 photosynthetic enzymes, PC, PK, ME and NADP-malate dehydrogenae (MDH), were determined by enzyme assay and western immuno-blotting, respectively. The photosynthetic rates of the 2nd upright leaf at the heading stage were measured at 30-32^oC using a LI-6200 portable photosynthesis system. A portable chlorophyll fluorometer (PAM-2000) was used to measure *Fv*, *Fm*, *q*N and *q*P on attached leaves before and after photo-oxidative treatment (high light and methyl viologen treatment).

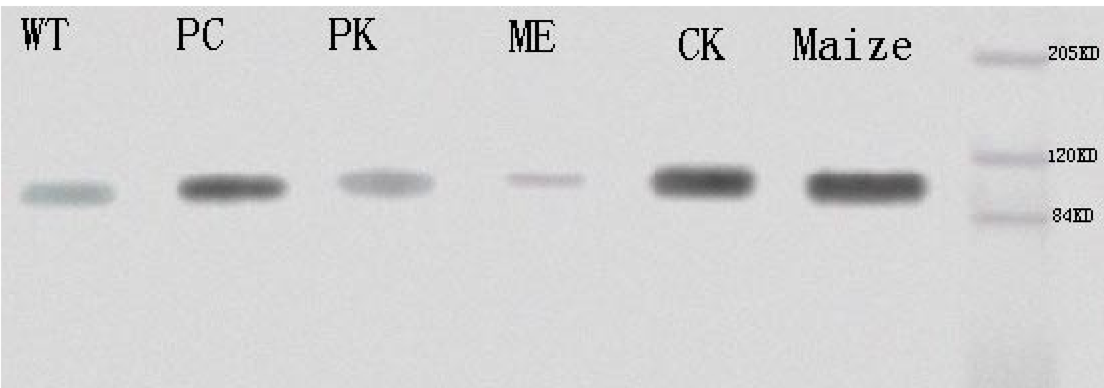
Results and Discussion

Expression of transgenes

The activities of PC, PK, ME and MDH in the various transgenic lines as well as untransformed rice were examined by direct assay of the enzymes (Figure 2) and by western immuno-blotting using specific antibodies (Figure 1). The activities and enzyme protein contents of the C_4 enzymes were very low in untransformed rice. In contrast, the activities of PC in PC transgenic rice were about 20-fold higher than that in untransformed rice reaching 1400 µmol/mg Chl/h, with a concomitant increase in protein amount (Ku et al. 1999). The activity of Rubisco and its kinetic property were not altered in the transgenic plants. Somewhat unexpected is the stimulation of CA activity in the PC transgenic rice, which increased by more than two fold, indicating a metabolic adaptation (Ku et al. 2000). The activities of ME in ME transgenic rice and the activities of PK in PK transgenic rice were 5 fold and 4 fold higher than those found in untransformed rice plant, respectively.

Photosynthetic characteristics

The photosynthetic characteristics of PC transgenic plants were analyzed in details. The plants exhibited higher light-saturated photosynthetic rates (55%, at 1200 µmol phton/m^2/s) and higher carboxylation efficiency (50%) than untransformed WT. The PC transgenic plants also had higher (20%) photosynthetic rates at optimal temperature (35^oC). On other hand, the photosynthetic CO_2 compensation points were slightly lower in the PC transgenic plants, indicating a stronger capability of the plants to assimilate carbon under limited CO_2 conditions. Taken together, these results suggest that the high PC coupled with enhanced CA confers the transgenic plants a higher capability to assimilate atmospheric CO_2. However, the exact mechanism for the superior photosynthetic performance of these plants remains to be determined, as there is only low amount of atmospheric CO_2 being directly fixed via the C_4 pathway (Ku et al. 2000).



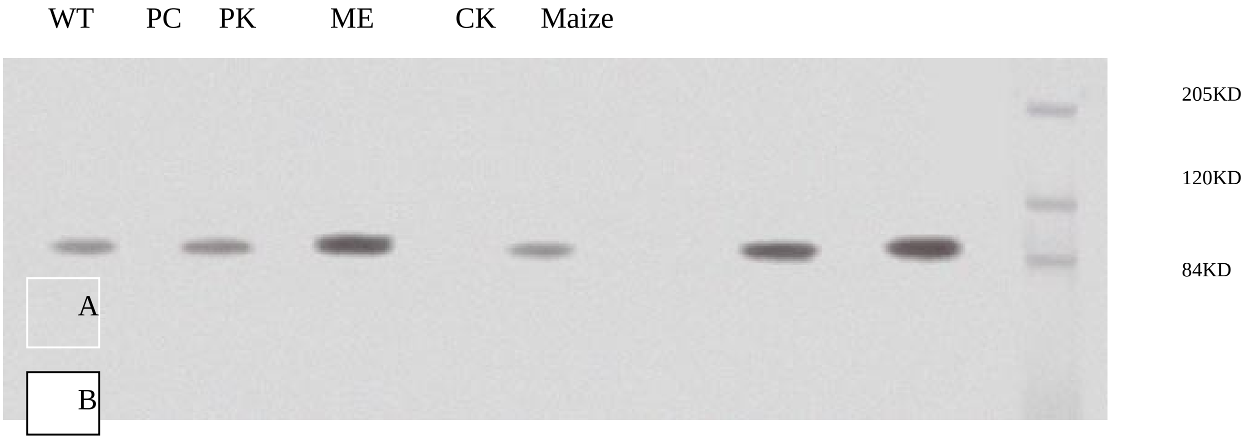


Fig.1. The western blot of PEPC (A) and PPDK (B) in C_4 photosynthesis enzymes transgenic rice plants and untransformed rice plants

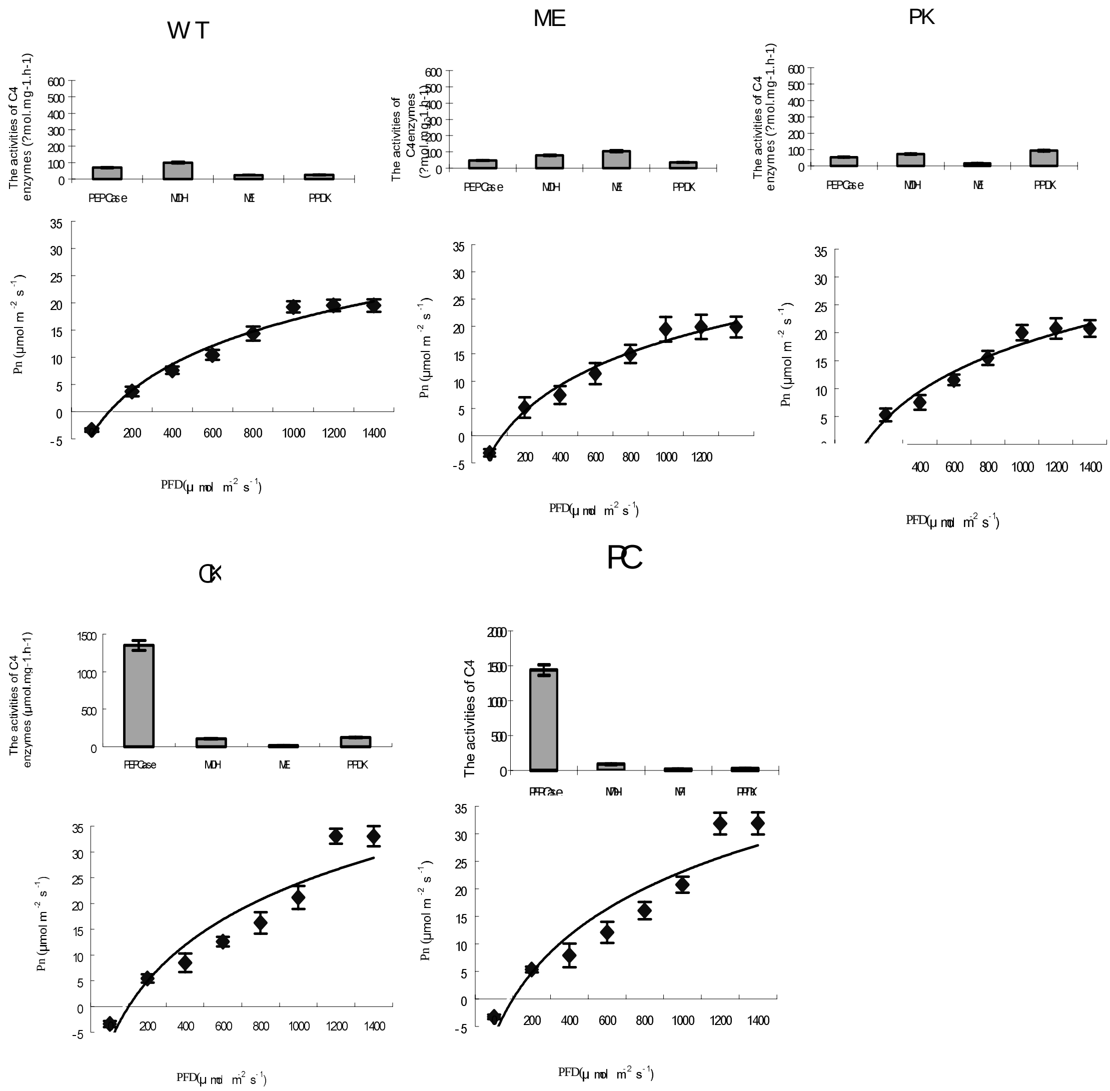


Fig.2. Light-photosynthesis curves in the flag leaves of C4 photosynthesis enzymes transgenic rice, untransformed rice and maize. WT indicates untransformed rice (kitaake); ME indicates NADP-ME transgenic rice; PK indicates PPDK transgenicrice; PC indicates PEPC transgenic rice; PCKindicates PEPC+PPDK transgenic rice; PFD indicates photon flux density.

Table 1 The activity of PC and some physiological parameters of photosynthesis of untransformed and PC transgenic rice plants. Values were means +/- SD from 10-12 measurements.

Genotype	Activity of PC (µmol/mg Chl/h)	Light-saturated photosyn-thetic rate (µmol/m²/s)	Photosynthetic rate at optimal temperature (µmol/m²/s)	Carboxy-lation efficiency (mol/mol)	CO_2 compensation point (µmol/mol)
Untrans-formed (WT)	69.7±5.8	20.34±0.98	23.98±1.07	0.077±.003	66.3±2.1
PC transgenic (PC)	1421 +/- 117	31.30±1.38	28.72±1.59	0.115±.005	52.2±1.3

Tolerance to photo-oxidation

To test if PC transgenic plants can better tolerate photoinhibition and photo-oxidative stress, the plants were treated with high light and methyl viologen (MV, an artificial oxidative reagent) plus high light conditions, respectively. Chlorophyll *a* fluorescence analysis indicates that the electron transport efficiency of PSII, as determined by *Fv/Fm*, decreases more in WT than in PC transgenic plants, especially after photo-oxidative treatment (36% vs. 16% inhibition). Consistently, the ability to dissipate the excess

Table 2. Changes of chlorophyll fluorescence parameters in PC transgenic rice

G	*F*v/*F*m LT	*F*v/*F*m HL	*F*v/*F*m HL+MV	Inhibi-tion (%)	*q*P LT	*q*P HL	*q*P HL+MV	Inhib-tion (%)	*q*N LT	*q*N HL	*q*N HL+MV	Incre ment- (%)
WT	0.84	0.75	0.48	35.7	0.85	0.79	0.58	26.7	0.47	0.43	0.44	1.1
PC	0.84	0.79	0.67	16.1	0.85	0.80	0.67	16.8	0.47	0.44	0.59	33.1

G, genotype; WT, untransformed rice; PC, PC transgenic rice; LT, sprayed with distilled water and exposed to low light (20-30 µmol /m²/s); HL, sprayed with distill water and exposed to high light (1400 µmol /m²/s); HL+MV, sprayed with 1.5 mM methyl viologen (MV) and exposed to high light (1400 µmol /m²/s).

light energy by photochemical means (*q*P) also decreased more after the photo-oxidative stress in the WT than in PC transgenic plants (27% vs. 17% inhibition). Interestingly, the ability to dissipate excess light energy via non-photochemical means (*q*N) in WT plants did not vary, while it increased by 33% in PC transgenic rice plants after photo-oxidative treatment. It is clear that under oxidative conditions the PSII electron transport system of PC transgenic rice plants exhibits a higher stability, presumably due to more effective dissipation of excessive light energy via photochemical and non-photochemical means. Therefore, PC transgenic rice is more tolerant to photo-oxidation and able to maintain a higher photosynthetic capacity under the condition.

Growth and grain yield

Among the five genotypes tested, PC and PC+PK transgenic rice showed significant increases in grain yield, being 22% and 24%, respectively. The increases in grain yield are mainly associated with increased panicle number per plant. No significant changes in seed weight were noticed. A more stable photosynthetic capacity may result in higher grain yield.

Table 3. The yield and its components in transgenic rice expressing C_4 photosynthesis enzymes.

Transgenic rice	WT	ME	PK	PC	PC+PK
Panicles per pot	38.5±1.9	39.8±1.7	41.5±2.1	44.5±1.8	45.1±2.0
Spikelets per panicle	37.6±2.1	37.6±2.6	37.7±1.7	37.8±1.9	38.0±2.2
Filled grains per panicle	28.5±2.0	28.8±2.3	29.0±2.7	29.9±1.9	30.2±2.1
Seed setting rate (%)	75.8	76.6	76.9	79.1	79.5
1000-grain weigh (g)	22.7	22.7	22.7	22.7	22.7
Yield per pot (g)	24.6±2.1	25.5±2.6	26.2±2.4	30.0±1.8	30.6±2.0

WT, untransformed rice; ME, ME transgenic rice; PK, PK transgenic rice; PC, PC transgenic rice; PC+PK, transgenic rice simultaneously expressing PC and PK.

Photosynthetic characteristics for hybrids rice with transgenic PEPC parent

Japonica rice cultivar 9516 and *indica* rice, restorer lines 9311, as the female parents were crossed with the sixth transgenic rice with maize *PEPC* gene, photosynthetic characteristics in the F_1 hybrids and parents were identified. The results showed: 505 hybrid plants were obtained and the PEPC activities among these plants exhibited normal distribution. The percentage of the plants with PEPC activity lying between two parents (100~400 μ mol PEP/mgChl.h) was 75.7% and that with high (400~880 μ mol PEP/mgChl.h) and low (lower than 100 μ mol PEP/mgChl.h) PEPC activity were 8.3% and 4.3%, respectively. 42 hybrid plants were screened and identified with high PEPC activity and high photosynthetic rate. The physiological indexes of the 42 plants were markedly superior to those of their female parents, increased 31.3% in saturated photosynthetic rate, and decreased 16.2%~17.6% in CO_2 compensation point. The correlation coefficient between PEPC activity and saturated photosynthetic rate (Pn) was 0.67**. In compared with their female parents, the photosynthetic rate of the selected plants was obviously high during a fine day, peculiarly exhibiting a slighter photoinhibition of photosynthesis at noon. It is suggested that the *PEPC* gene in transgenic rice might be incorporated into the rice plant used. The superior transgenic rice plants have been obtained by pollen culture. At present, the plant lines were identified to select and develop into stable cultivar lines in the field. So, Based on the present good plant-type and heterosis, that C_4 photosynthetic genes introduced into high-yield rice cultivur through genetic manipulation would open up a new approach to rice breeding.

Acknowledgements

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