

Photosynthetic performance of transgenic rice plants overexpressing maize C₄ photosynthesis enzymes

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Transgenic rice plants overexpressing maize C₄-specific phosphoenolpyruvate carboxylase (PEPC) exhibit a higher photosynthetic rate (up to 30%) and a more reduced O₂ inhibition of photosynthesis than untransformed plants. There is a small increase in the amount of atmospheric CO₂ being directly fixed by PEPC. Similarly, transgenic rice plants overexpressing the maize chloroplastic pyruvate, orthophosphate dikinase (PPDK), also have higher photosynthetic rates (up to 35%) than untransformed plants. This increased photosynthetic capacity is at least in part due to an enhanced stomatal conductance and a higher internal CO₂ concentration. Using conventional hybridization, we have integrated maize PEPC and PPDK genes into the same transgenic rice plants. In the segregating population, the photosynthetic rates of plants with high levels of both maize enzymes are up to 35% higher than those of untransformed plants.

Under full-sunlight conditions, the photosynthetic capacity of field-grown PEPC transgenic rice plants is twice as high as that of untransformed plants. PEPC transgenic plants consistently have a higher photosynthetic quantum yield by photosystem II and a higher capacity to dissipate excess energy photochemically and nonphotochemically. Preliminary data from field tests show that the grain yield is about 10–30% higher in PEPC and 30–35% higher in PPDK transgenic rice plants relative to untransformed plants. Taken together, these results suggest that introduction of C₄ photosynthesis enzymes into rice has a good potential for enhancing the crop's photosynthetic capacity and yield.

Due to the CO₂-concentrating mechanism in the C₄ pathway of photosynthesis, C₄ plants have many desirable agronomic traits, such as high photosynthetic capacity and high mineral-use efficiency, especially under high light, high temperature, and drought conditions (Hatch 1987, Ku et al 1996). On the other hand, plants that assimilate atmospheric CO₂ via the C₃ pathway, including many agronomically important species such as rice, suffer from O₂ inhibition of photosynthesis and the associated photorespiration, and thus exhibit a lower photosynthetic efficiency under these conditions. Conventional hybridization has been employed to transfer C₄ traits to C₃ plants (see Brown and Bouton 1993); however, epistatic interaction between the alleles suppresses the expression of C₄ traits in the progeny, and genes for Kranz leaf anatomy and biochemistry of C₄ photosynthesis are not closely linked. Most importantly, no closely related C₃ and C₄ crops can be hybridized. Several attempts have been made in the past to transfer the genes involved in C₄ photosynthesis into C₃ plants (Hudspeth et al 1991, Kogami et al 1994, Gehlen et al 1996, Gallardo et al 1995, Ishimaru et al 1998) in an effort to tune up

their photosynthetic metabolism. However, limited physiological consequences were observed in these transgenic plants, which may be due to the low levels of expression of these genes.

Using an *Agrobacterium*-based transformation system, we have independently introduced three key C₄ photosynthesis genes from maize into rice with high levels of expression (Agarie et al 1998, Ku et al 1999). These are phosphoenolpyruvate carboxylase (PEPC), pyruvate, orthophosphate dikinase (PPDK), and NADP-malic enzyme (NADP-ME). The major objective of our research is to introduce enzymes involved in C₄ photosynthesis into C₃ plants and test their effects on photosynthesis and plant productivity. By introducing some of the key enzymes of C₄ photosynthesis into C₃ plants with proper intercellular compartmentation, a limited C₄ acid metabolism may be installed for fixing atmospheric CO₂ directly via this pathway and partially concentrating CO₂ in the chloroplast. In this regard, a similar mechanism has been found in the primitive aquatic angiosperm *Hydrilla verticillata* (Magnin et al 1997). The photosynthetic mechanism in *H. verticillata* is considered a primitive form of C₄ photosynthesis. When it is grown under low CO₂ conditions, it shifts from C₃ to C₄ photosynthesis and assimilates atmospheric CO₂ via the C₄ pathway without Kranz leaf anatomy (Bowes and Salvucci 1989). Inorganic carbon is first assimilated into the C₄ acid malate in the cytoplasm via PEPC. Subsequently, malate serves as a donor of CO₂ to Rubisco in the chloroplast by the decarboxylating enzyme NADP-ME. This primitive-type C₄ photosynthesis is sufficient to concentrate CO₂ in the chloroplast and overcome photorespiration (Reiskind et al 1997). It is possible that this archetypal version of C₄ photosynthesis, which does not depend on Kranz compartmentation, can be engineered to function in terrestrial C₃ plants.

Enhanced expression of enzymes of C₄ photosynthesis in C₃ plants may increase carbon and nitrogen metabolism in certain tissues of C₃ plants. All enzymes involved in C₄ photosynthesis are found in leaves of C₃ plants. Although they are low in activity in leaves of C₃ plants, some of them are found at high levels in reproductive tissues. For example, the cytosolic isoform of PPDK occurs at high levels in seeds of both the C₃ plant wheat (Aoyagi and Bassham 1984a,b, Aoyagi and Chua 1988, Blanke and Lenz 1989) and the C₄ plant maize (Imaizumi et al 1997). Thus, PPDK may play an important role in linking carbon and nitrogen metabolism or supply of energy (e.g., release of ATP from PEP catalyzed by PPDK) in reproductive tissues, and enhanced expression of the enzyme may boost seed development and grain productivity.

Enzymes involved in C₄ photosynthesis, although low in C₃ plants, may also play important roles in plant defense responses to biotic and abiotic stress. Metabolic alterations in response to stress allow plants to adapt to adverse conditions. For example, an increase in NADP-ME by wounding, low oxygen, low temperature, salinity, and ultraviolet light has been reported in C₃ plants such as rice (Fushimi et al 1994) and bean (Walter et al 1994, Schaaf et al 1995, Pinto et al 1999) and the C₄ plant maize (Drincovich et al 1998). It is postulated that the reductant (NADPH) released from decarboxylation of malate by NADP-ME may be required for the increased synthesis of secondary metabolites for defense purposes. Furthermore, increased expression of PPDK in C₃ chloroplast may enhance synthesis of aromatic amino acids, such as phenylalanine via the shikimic pathway (Hermann 1995), which serve as the substrate for secondary metabolites (e.g., phenylpropanoids), which are part of the biosynthesis involved in plant defense mechanisms (Douglas 1996). The biosynthesis of phenylpropanoids requires the efficient flow of carbon into phenylalanine biosynthesis. Thus, increased expression of some C₄ photosynthesis enzymes in C₃ plants could confer enhanced tolerance under stress conditions.

In this chapter, we report on the photosynthetic traits of transgenic rice plants that express maize C₄-specific PEPC and PPDK independently. In addition, the photosynthetic performance of hybrid transgenic plants overexpressing both maize PEPC and PPDK was also measured.

This represents a first step in engineering the biochemical components of C_4 photosynthesis in rice.

Transgenic rice plants overexpressing maize PEPC

In previous studies (Agarie et al 1998, Ku et al 1999), we reported that the primary transgenic rice plants harboring the maize PEPC gene have high levels of expression (up to 12% of total leaf soluble protein) and the enzyme remains active. Immunolabeling studies indicate that the enzyme is localized in the cytosol of mesophyll cells (data not shown). Genetic studies show that the maize gene is stably inherited in a Mendelian manner, with the gene being inserted at one or two loci. Furthermore, the photosynthetic rates, measured under ambient conditions, of these transgenic plants are comparable with or higher than those of untransformed plants (Ku et al 1999). In addition, O_2 inhibition of photosynthesis decreases progressively with an increasing level of PEPC activity among the transgenic plants. Our preliminary labeling experiments with $^{14}CO_2$ in leaves shows only a small increase (4%) in atmospheric CO_2 being directly fixed by PEPC. The supply of PEP, the substrate for PEPC, may be limited in C_3 leaf. Thus, the biochemical and physiological bases of these alterations in photosynthetic traits remain unclear.

Using the segregating populations from four primary transgenic lines that exhibit high levels of maize PEPC, we have shown that the photosynthetic rates (on a leaf area basis) of flag leaves in most PEPC transgenic rice plants are either similar to or up to 30% higher than those of untransformed plants (average $17.5 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$, Fig. 1A). The photosynthetic rate begins to decrease as the level of expression reaches very high values, as one would expect. Indeed, transgenic plants with extremely high levels of PEPC have lower chlorophyll contents. Analysis of the relationship between photosynthetic rate and stomatal conductance among these plants shows a good positive correlation between the two parameters (Fig. 1B). Furthermore, stomatal conductance is highly correlated with intercellular CO_2 concentration (Fig. 1C). The intercellular CO_2 concentration in some transgenic plants is as high as $275 \mu\text{L L}^{-1}$ versus $235 \mu\text{L L}^{-1}$ in untransformed plants. Thus, part of the higher photosynthetic capacity of the transgenic plants may be due to the ability of the plants to maintain a higher internal CO_2 in the leaf because of increased stomatal opening. The immediate benefit of a higher intercellular CO_2 for the plants is elevated net carbon fixation due to suppression of Rubisco oxygenase and photorespiration. We also observed a consistent upward shift in optimal temperature for photosynthesis by the transgenic plants from 26 to 28–32 °C (data not shown), presumably because of reduced photorespiration. Interestingly, the improvement in wheat grain yield in the past 30 years by CIMMYT (27%) is mainly due to an increased stomatal conductance in the new cultivars (63%), which results in a 23% increase in maximum photosynthetic rate (23%) and a canopy temperature depression of 0.6 °C (Fischer et al 1998). Furthermore, ^{13}C discrimination is also positively correlated with yield progress.

Consistent with the suggestion that PEPC transgenic rice plants have a higher stomatal conductance than untransformed wild-type plants, the $\delta^{13}C$ values for the transgenic plants are 1.5–2.5‰ more negative than that of untransformed plants (–27.5‰) and the value becomes more negative with increasing PEPC activity among the transgenic plants (Fig. 2). It has been shown that an increased stomatal conductance allows more CO_2 to diffuse into the leaf and thus more ^{13}C is discriminated during photosynthesis (Winter et al 1982). However, the possibility that the lower ^{13}C content in the leaves of transgenic plants could be due to refixation of photorespiratory CO_2 by PEPC and then Rubisco again cannot be ruled out. The interesting question here is how transgenic plants manage to maintain a higher stomatal conductance. The

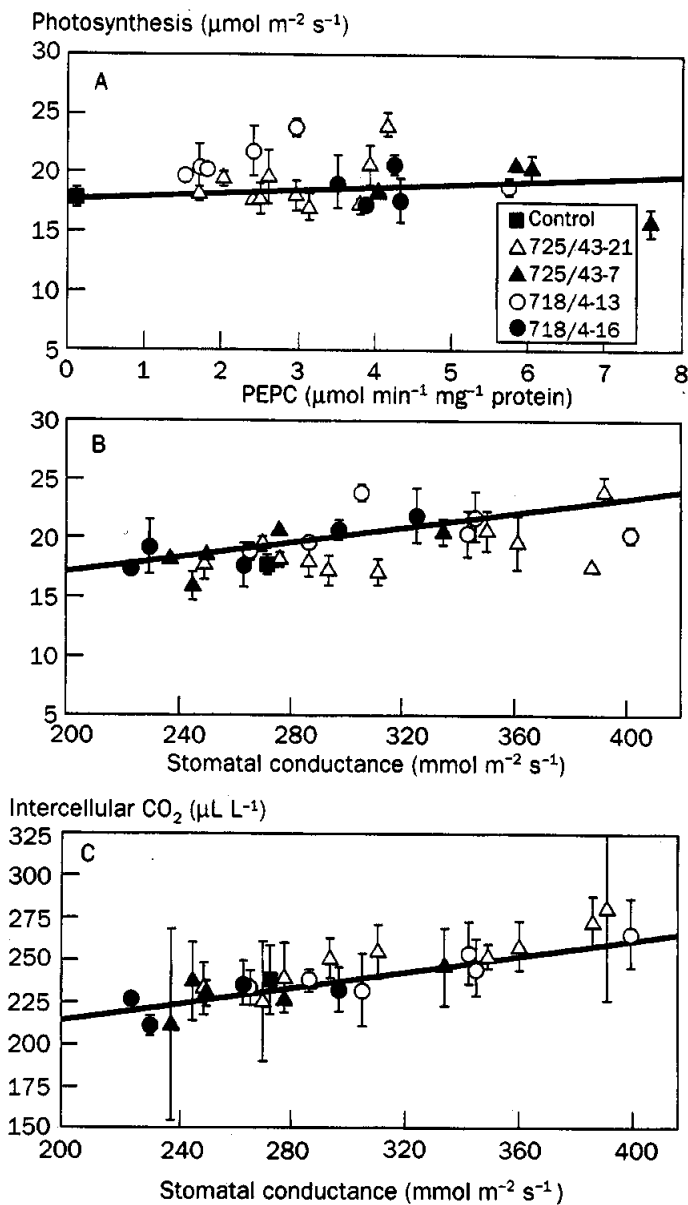


Fig. 1. (A) Photosynthetic rate as a function of PEPC activity, (B) relationship between photosynthetic rate and stomatal conductance, and (C) relationship between stomatal conductance and intercellular CO₂ concentration in untransformed control (Kitaake) and PEPC transgenic rice plants. Transgenic plants generated from segregating populations of four independent T₁ plants were used for analysis. Photosynthesis was measured in newly matured flag leaves at 26 °C, 1,200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ and 360 $\mu\text{L L}^{-1}$ CO₂. Unpublished data of D. Cho, U. Ranade, and M.S.B. Ku.

mechanism underlying this phenomenon is not quite clear. It is conceivable, however, that an increased expression of PEPC in the guard cells would allow more fixation of atmospheric CO₂ into organic acids such as malate, which are stored in the vacuole. Consequently, inorganic solutes such as potassium move from subsidiary or epidermal cells into guard cells for balance of charge. The accumulation of ions in the vacuole lowers the water potential of the guard cell, thereby stimulating the osmotic uptake of water and increased turgor for opening of stomates.

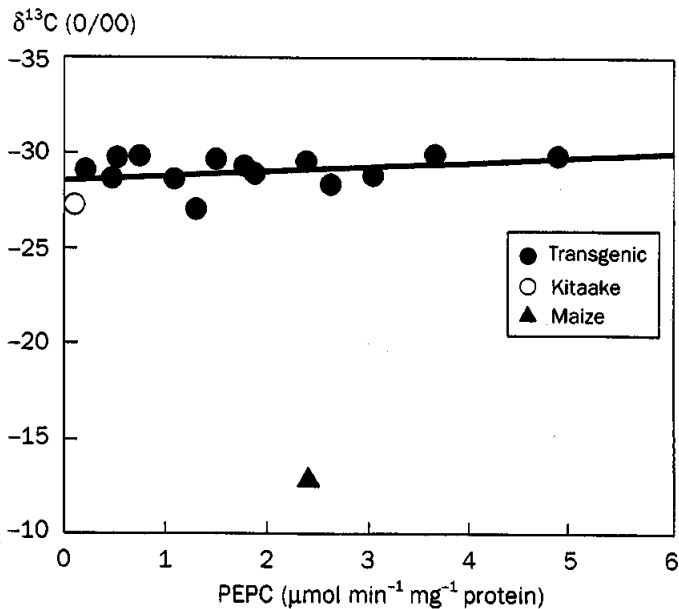


Fig. 2. Leaf $\delta^{13}\text{C}$ value as a function of PEPC activity for maize, untransformed control (Kitaake), and PEPC transgenic rice plants. Newly mature flag leaves from primary transgenic (T_0) plants were used for measurement of ^{13}C content. Unpublished data of J. Ehleringer and M.S.B. Ku.

PEPC transgenic rice plants grown in the field also exhibit a better photosynthetic performance than untransformed plants. The photosynthetic rate of untransformed plants is saturated by half full sunlight, whereas that of PEPC transgenic plants does not show saturation until full sunlight is reached. In one experiment, the light-saturated photosynthetic rates in PEPC transgenic plants ranged from 21.0 to $27.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, whereas the rates in untransformed plants were around $17.5 \mu\text{mol m}^{-2} \text{s}^{-1}$. These results suggest that the leaves of PEPC transgenic plants are capable of using full sunlight to maximize carbon gain. On the other hand, photosynthesis by untransformed plants is inhibited by high light (photoinhibition). Consistent with this observation, the intrinsic quantum yield of photosystem II (PSII) as measured by F_v/F_m is less inhibited by full-sunlight treatment alone or by a combination of methyl viologen and full-sunlight treatment in PEPC transgenic plants relative to wild-type plants (Fig. 3). Methyl viologen accepts electrons from PSI and generates oxy-radicals. Under these high-light and photooxidative conditions, PEPC transgenic plants are capable of dissipating excess light energy through photochemical and nonphotochemical means more effectively than untransformed plants, as demonstrated by the measurements of q_P (photochemical quenching) and q_N (nonphotochemical quenching), respectively. Taken together, these results indicate that PEPC transgenic plants are less susceptible to photoinhibition or photooxidation, which may contribute to the increased photosynthetic capacity. The basis for the superior ability of PEPC transgenic plants to dissipate excess light energy is not known at present.

It is also quite possible that overexpression of the maize C_4 PEPC in the transgenic rice plants (up to 12% in primary transgenic plants, Ku et al 1999) may influence the activities of other photosynthetic enzymes or enzyme kinetics. This needs to be evaluated in relation to the photosynthetic performance of these transgenic plants. In this experiment, the chlorophyll contents were similar between untransformed plants and the transgenic rice plants used. On a chlorophyll basis, Rubisco activities in untransformed and transgenic plants are similar (340

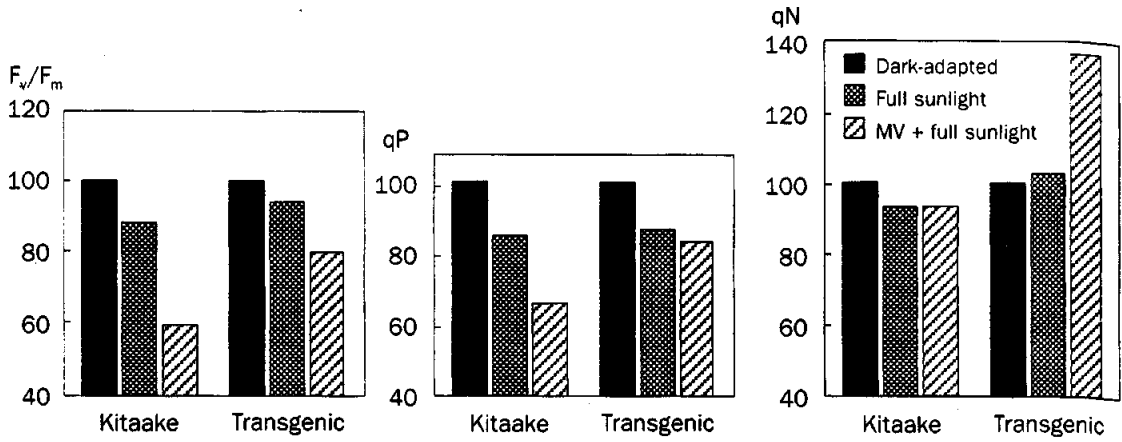


Fig. 3. Intrinsic quantum yield of PSII (F_v/F_m), photochemical quenching (qP), and nonphotochemical quenching (qN) for dark-adapted leaves, leaves illuminated for 3 h under full sunlight, or leaves treated with 1.5 mM methyl viologen (MV) and then illuminated for 3 h under full sunlight in untransformed control (Kitaake) and PEPC transgenic plants. Plants were grown in pots and maintained outdoors during May–August in Nanjiang, China (1999), and newly mature flag leaves and the leaves below were used for measurements. Unpublished data of X. Li, D.-M. Jiao, and M.S.B. Ku.

Table 1. Activities of PEP carboxylase (PEPC), carbonic anhydrase (CA), and Rubisco, and kinetics of Rubisco in PEPC transgenic and untransformed (Kitaake) rice plants. Plants were grown in pots and maintained outdoors during May–August (Nanjiang, China, 1999); newly mature flag leaves and the leaves below were used for enzyme extraction after illumination at $1,400 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ for 4–6 h. Enzymes were assayed at 30°C and the data for enzyme activity were means \pm standard deviation from 3–6 replicates of measurements. Unpublished data of X. Li, D.-M. Jiao, and M.S.B. Ku.

Enzyme	Kitaake	Transgenic
PEPC ($\mu\text{mol mg}^{-1} \text{Chl h}^{-1}$)	165 ± 9	$1,265 \pm 66$
CA ($\mu\text{mol mg}^{-1} \text{Chl h}^{-1}$)	204 ± 11	577 ± 28
Rubisco ($\mu\text{mol mg}^{-1} \text{Chl h}^{-1}$)	340 ± 24	367 ± 21
Rubisco $K_m(\text{CO}_2)$ (μM)	11.95	11.53
Rubisco V_{max} ($\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$)	2.38	4.77

vs $367 \mu\text{mol mg}^{-1} \text{Chl h}^{-1}$) (Table 1). Also, the $K_m(\text{CO}_2)$ of Rubisco is the same between the two plants (12.0 vs $11.5 \mu\text{M}$). However, the V_{max} of Rubisco on a protein basis is twice as high in PEPC transgenic plants as in untransformed plants (4.77 vs $2.38 \mu\text{mol mg}^{-1} \text{protein min}^{-1}$). In addition, carbonic anhydrase (CA) is almost three times higher in the transgenic plants (577 vs $204 \mu\text{mol mg}^{-1} \text{Chl h}^{-1}$). These results suggest that enhanced expression of the maize PEPC in rice may have altered the expression or activation state of other photosynthetic enzymes in the leaves. Similarly, transgenic tobacco overexpressing a gene that encodes chloroplast-localized Cu/Zn superoxide dismutase (3-fold) also exhibits a 3–4-fold increase in ascorbate peroxidase because of increased transcription (Gupta et al 1993). This phenomenon warrants further investigation. In any case, increased CA activity may enhance the fixation of atmospheric CO_2 via PEPC as HCO_3^- is the active CO_2 species for PEPC (Hatch 1987). The higher V_{max} of Rubisco in the leaves of transgenic plants may compensate for its lower amount (as a percentage) because of overexpression of the maize PEPC (Ku et al 1999) and thus allow the plants to maintain a high photosynthetic capacity.

Transgenic rice plants overexpressing maize PPDK

The primary transgenic rice plants harboring the maize chloroplast PPDK gene exhibit a wide range of enzyme activity, up to 10-fold higher than that in untransformed plants (Agarie et al 1998). Studies with isolated chloroplasts from leaves of PPDK transgenic plants indicate that the majority of PPDK in the mesophyll cell is localized in the chloroplast. The photosynthetic performance of PPDK transgenic plants was evaluated using segregating populations from four primary transgenic lines that exhibit high levels of PPDK. As expected, the amount of PPDK varies in segregating populations (Fig. 4). Most of the PPDK transgenic plants exhibit a higher photosynthetic rate (up to 35%) than the wild-type plants, and the higher photosynthetic rates are associated with increased stomatal conductance (Fig. 5) and higher intercellular CO₂ concentration (data not shown), similar to those found in the PEPC transgenic plants. Thus, as with PEPC transgenic plants, PPDK transgenic rice plants may also be able to maintain a

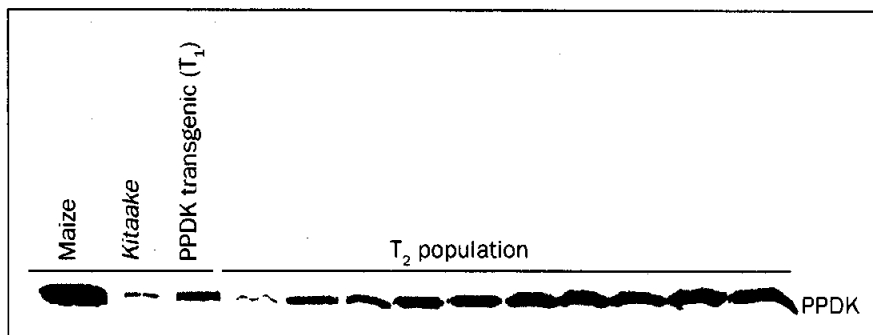


Fig. 4. Immunoblot of PPDK in leaves of maize, untransformed control (Kitaake), and a primary (T₁) PPDK transgenic rice plant and its segregating population. Unpublished data of T-P. Hsu and M.S.B. Ku.

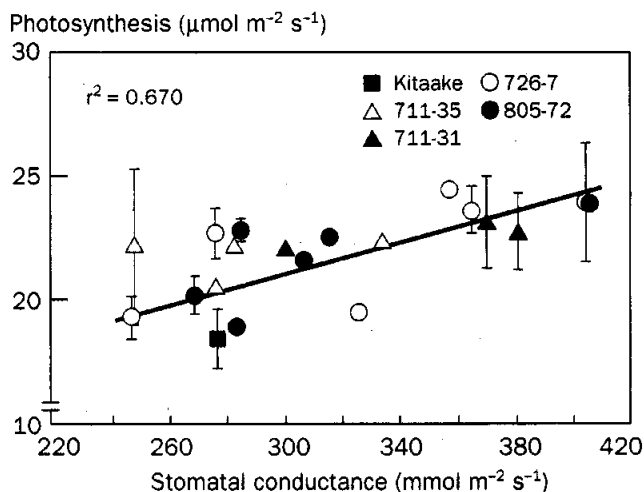


Fig. 5. Relationship between photosynthetic rate and stomatal conductance among untransformed control plant (Kitaake) and segregating populations from four primary (T₀) PPDK transgenic rice plants. Measurement conditions were the same as described in Fig. 1. Unpublished data of D. Cho and M.S.B. Ku.

higher internal CO₂ level due to increased stomatal conductance. Increased expression of PPDK in the guard cells may function to supply PEP, the substrate for PEPC, for synthesis of organic acids. How the elevated PPDK may affect carbon and nitrogen metabolism in leaves of transgenic rice plants awaits further investigation.

The effects of elevated expression of maize PPDK on carbon metabolism in transgenic potatoes (C₃) have been reported recently (Ishimaru et al 1998). PPDK activities in leaves of transgenic potatoes are up to 5-fold higher than those of untransformed control plants. Analysis of metabolites shows that PPDK activity in leaves is negatively correlated with pyruvate content and positively correlated with malate content. It is suggested that elevated PPDK activity in the leaf may lead to a partial function of C₄-type carbon metabolism. However, the altered carbon metabolism does not have any significant effect on other photosynthetic characteristics.

Transgenic rice plants overexpressing both maize PEPC and PPDK

Since PEPC catalyzes the initial fixation of atmospheric CO₂ in the C₄ pathway and PPDK catalyzes the conversion of pyruvate to PEP, overexpression of both enzymes simultaneously may enhance the fixation of atmospheric CO₂ via PEPC. Using conventional hybridization, we have integrated both maize PEPC and PPDK genes into the same plants from two independent homozygous transgenic rice plants that express high levels of the maize enzymes. The amounts of the two enzymes in the F₁ hybrids are about half of those in the parents (Fig. 6). The photosynthetic performance of the transgenic plants expressing varying amounts of the two maize enzymes was first evaluated in the segregating population from one of the F₁ hybrids. As expected, the segregating population exhibits different combinations for the amounts of the two enzymes, with some combinations having only the same amount as the wild-type plants (without the maize gene inserted) and others having twice the amount of the parental transgenic plants (homozygous with respect to the inserted maize gene) (Fig. 7). The activities of each enzyme are well correlated with the amounts of the protein. Although the overall photosynthetic rates in this experiment are somewhat lower than those obtained earlier due to cultivation in smaller pots, the photosynthetic trend for the activities of the two maize enzymes is clear (Fig. 8). Hybrid plants expressing high levels of both PEPC and PPDK tend to have a higher photosynthetic rate (up to 35%) than untransformed plants, again because of higher stomatal conductance and higher intercellular CO₂ (data not shown). It is quite possible that overexpression of both enzymes further enhances the capability of the plants to synthesize organic acids in the guard cells and consequently the conductance of CO₂ into the leaf. With two of the key enzymes of the C₄ pathway introduced into the same plants, experiments are under way to see if the leaves of these plants are capable of fixing more atmospheric CO₂ directly via this route.

Future directions

In summary, our physiological results demonstrate that introduction of maize PEPC and PPDK into rice has the potential to enhance its photosynthetic capacity by increasing stomatal conductance for CO₂ diffusion. PEPC transgenic rice plants are also more tolerant of photoinhibition and photooxidation under field conditions. This trait is important for rice productivity as early senescence of leaves, due to photoinhibition and photooxidation, often occurs in the field, which reduces grain yield. The performance of these transgenic plants under other stress conditions such as water deficit, high and low temperatures, and mineral deficiency needs to be evaluated in the future. The higher stomatal conductance exhibited by

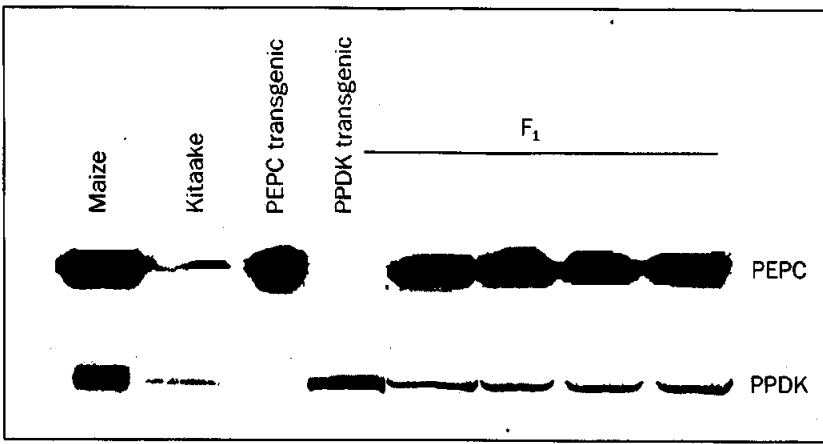


Fig. 6. Immunoblot of PEPC and PPDK in leaves of maize, untransformed control (Kitaake), PEPC, and PPDK transgenic rice plants, and four F₁ hybrids between PEPC and PPDK transgenic plants. Unpublished data of T-P. Hsu, D. Cho, and M.S.B. Ku.

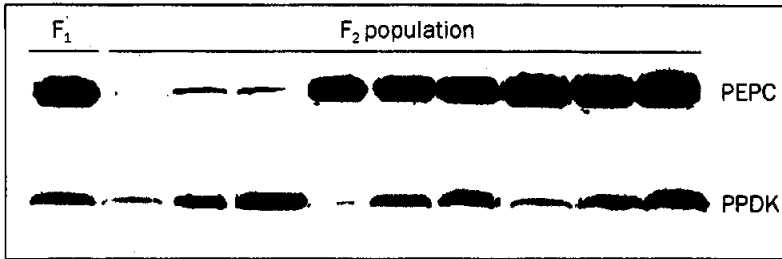


Fig. 7. Immunoblot of PEPC and PPDK in leaves of an F₁ hybrid between PEPC and PPDK transgenic rice plants and the F₂ population. Unpublished data of T-P. Hsu, D. Cho, and M.S.B. Ku.

the transgenic rice plants implies that more water may be needed. However, this may not be a serious problem for paddy rice. A preliminary small-scale test in the field shows that the grain yield is about 10–30% higher in PEPC and 30–35% higher in PPDK transgenic rice plants relative to untransformed plants, in spite of a lower fertility (5–10%). The increases in grain yield are mainly associated with an increased number of panicles per plant (15–30%). These results suggest that these transgenic plants can perform well in variable environments. More field tests, especially on a large scale, will be required to confirm this. Also, whether this trait will be stably inherited in the following generations needs to be evaluated too.

Transgenic rice plants overexpressing PEPC, PPDK, or both may not be capable of fixing a large amount of atmospheric CO₂ directly as in C₄ plants due to a limited supply of substrates or further metabolism of products. However, with the introduction of another key enzyme of the C₄ pathway, NADP-ME in the chloroplast, a limited CO₂-concentrating mechanism may be achieved. Enhanced expression of other biochemical components of the C₄ pathway, such as CA, NADP-malate dehydrogenase, adenylate kinase, and transporters specific for C₄ metabolites, may allow the cycle to function more effectively. In this regard, the increased activities of CA and Rubisco in PEPC transgenic rice plants are worth noting; some related enzymes in the pathway may be induced or enhanced. This raises an interesting question on metabolic adaptation, and more studies on expression of related genes in the transgenics will

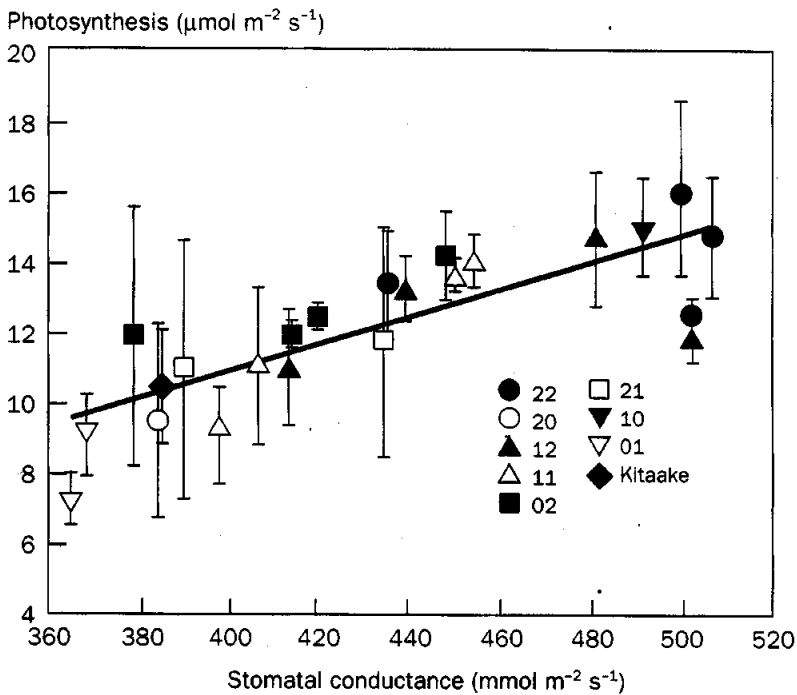


Fig. 8. Relationship between photosynthetic rate and stomatal conductance among untransformed control plant (Kitaake) and the F₂ population from an F₁ hybrid between PEPC and PPK genes transgenic rice plants. Measurement conditions were the same as described in Fig. 1. Unpublished data of D. Cho and M.S.B. Ku. Designation of plants: "22" represents plants with homozygous maize PEPC and PPK genes and "21" represents plants with homozygous maize PEPC and heterozygous maize PPK genes, while "20" represents plants with homozygous maize PEPC and no maize PPK gene, and so on.

be needed to address this issue. As discussed earlier, a primitive C₄-type photosynthesis has been reported to function in the aquatic angiosperm *H. verticillata* without Kranz anatomy (Magnin et al 1997). Perhaps a similar system can be engineered in terrestrial C₃ plants to concentrate CO₂ in the leaf and reduce carbon loss from photorespiration. A more efficient CO₂-concentrating mechanism by the C₄ pathway would require the concomitant installation of Kranz leaf anatomy. At present, little is known about the biochemical processes or genes regulating the differentiation of Kranz leaf anatomy in C₄ plants.

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About the cover: Upper left is a photomicrograph of a transverse section of a rice leaf. The bulk of the leaf, the mesophyll (M), is composed of arm-cells with projections of the cell wall into the cell. The larger vascular bundles (dense yellow) have bundle sheath (BS) cells around them. Rice plants appear in upper right, maize plant in lower left. Lower right is a transverse section of a maize leaf. Three vascular bundles appear (dense yellow), surrounded by large BS cells. The M cells are arranged so that almost every M cell is in contact with a BS cell. This is known as Kranz anatomy, characteristic of terrestrial plants with C₄ photosynthesis. (Images of leaf sections by W.C. Taylor and Celia Miller, plant photos by A. Javellana.)