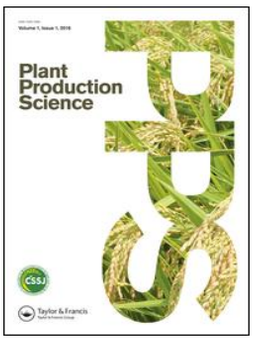

Plant Production Science

ISSN: 1343-943X (Print) 1349-1008 (Online) Journal homepage: https://www.tandfonline.com/loi/tpps20

Overexpression of C_4 Phospho*enol*pyruvate Carboxylase Increased Carbon Isotope Discrimination in Transgenic Rice Plants

Sakae Agarie, Haruto Sasaki, Makoto Matsuoka & Mitsue Miyao-Tokutomi

To cite this article: Sakae Agarie, Haruto Sasaki, Makoto Matsuoka & Mitsue Miyao-Tokutomi (2001) Overexpression of C_4 Phospho*enol*pyruvate Carboxylase Increased Carbon Isotope Discrimination in Transgenic Rice Plants, Plant Production Science, 4:4, 311-312, DOI: 10.1626/pps.4.311

To link to this article: https://doi.org/10.1626/pps.4.311

Plant Prod. Sci. 4(4) : 311—312 (2001)

[Short Report]

Overexpression of C_4 Phospho*enol*pyruvate Carboxylase Increased Carbon Isotope Discrimination in Transgenic Rice Plants

Sakae Agarie*[1], Haruto Sasaki*[2], Makoto Matsuoka*[3] and Mitsue Miyao-Tokutomi*[4]

(*[1] *Faculty of Agriculture, Saga Universiry, Saga 840-8502, Japan;* *[2] *Graduate School of Agriculture and Life Science, University of Tokyo, Yayoi, Bunkyo, Tokyo 113, Japan;* *[3] *BioScience Center, Nagoya University, Chikusa, Nagoya 464-8601, Japan;* *[4] *National Institute of Agrobiological Resources, Tsukuba 305-8602, Japan*)

Key words : Carbon Isotope Discrimination, C_3, C_4, Gene Manipulation, PEPC.

During photosynthetic CO_2 fixation, plants discriminate against the naturally occurring stable isotope $^{13}CO_2$. The overall discrimination is mainly determined by the differential diffusion of $^{13}CO_2$ and $^{12}CO_2$ during diffusion through stomata and the fractionations associated with the carboxylation enzymes. C_3 and C_4 plants can be distinguished by their carbon isotope composition of dry matter, because phospho*enol*pyruvate carboxylase (PEPC), a primary enzyme of atmospheric CO_2 fixation in C4 plant, discriminates against $^{13}CO_2$ much less than ribulose-1,5-bisphosphate carboxylase (Rubisco) (Park and Epstein, 1960). The carbon isotope discrimination (Δ) has been used in numerous studies to characterize responses of photosynthesis and growth to drought, salinity and other environmental stresses (reviewed by Farquhar et al.,1989 and references cited there in). In these studies, the isotopic composition of plant material was interpreted as representing a time-integrated record of CO_2 fixation dynamics in plants. We have used this method here to investigate the physiological impact of overexpressed C_4 PEPC on carbon metabolism in transgenic C_3 plants.

To introduce the high capacity of CO_2 fixation of C_4 plants into C_3 plants, several attempts have been made (Gehlen et al.,1996; Hausler et al., 1999; Hudspeth et al., 1991; Kogami et al.,1994). However, the activities of C_4 photosynthetic enzymes in transgenic C_3 plants were much lower than those in C_4 plants, and no significant impact on photosynthetic characteristics was observed. We have obtained transgenic rice plants expressing C_4 PEPC at comparable or even higher levels than in maize leaves (Ku et al., 1999). We have shown here that Δ was altered by the high level expression of PEPC in the transformants.

Transgenic plants were obtained as described by Ku et al. (1999). The plants were grown in a naturally illuminated greenhouse maintained at 27°C/22°C (day/night). Levels of expression of the introduced PEPC in transgenic rice were determined by assaying PEPC activity in leaf protein extracts as described by Ku et al. (1999). PEPC activity was determined at saturating PEP (5 mM) and optimal pH (pH 8.0) by the method of Slack and Hatch (1967). The assay was carried out at 30°C and activities were calculated on a protein basis. Adjacent portions of leaf tissues used for the PEPC assay were dried at 80°C and ground to a fine powder with a mortar and pestle, and the ^{13}C content was determined according to the method of Sasaki et al. (1996). The carbon isotope discrimination (Δ) can be expressed by the following equation (Hubick et al., 1986) :

$$\Delta = \frac{\sigma_a - \sigma_p}{1 + \sigma_a}$$

where σ_a and σ_p are the relative concentrations of ^{13}C in the atmospheric CO_2 and in the leaf tissues, respectively. We assumed a value of -8.0 per mil (‰) for the σ_a. This value is widely used for free atmospheric CO_2 (Farquhar et al., 1989). $^{13}CO_2$ was determined with a mass spectrometer (MAT-252, Finnigan MAT, San Jone, CA) as reported by Sasaki et al. (1996).

The extractable leaf PEPC activity in the transformants ranged from 14 to 80 times that in the nontransformants, and was 1-2 times that found in maize leaves (Fig. 1). Δ was increased with elevated PEPC activity in the transformants, there was a statistically significant difference ($P<0.05$) in Δ between nontransformants and transformant, indicating that $^{13}CO_2$ was more discriminated in the transformants with higher PEPC activities. This also suggested that the high PEPC activity influenced carbon metabolism in the transformants. This is the first report demonstrating that increased C_4 PEPC activity altered carbon isotope discrimination in a transgenic C_3 plant.

C_4 leaves always have a relatively low Δ value (approximately 2.0 to 10.2), because PEPC does not discriminate strongly between $^{13}CO_2$ and $^{12}CO_2$ in the mesophyll cells. In the transformants, however, although

Received 14 December 2000. Accepted 18 April 2001. Corresponding author : S. Agarie (agarie@cc.saga-u.ac.jp, fax +81-952-28-8736). This work was supported in part by a grant at the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN) to M.M.-T. and M.M. and from the Bio-Oriented Technology Research Advancement Institution (BRAIN) of Japan.

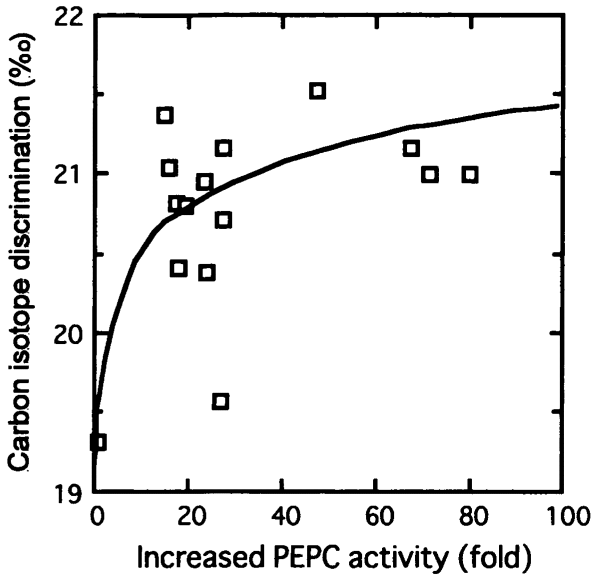

Fig. 1. Carbon isotope discrimination (Δ) for non-transformants and PEPC transgenic rice plants as a function of PEPC activity. PEPC activities ranged from 0.22 to 1.2 μmol mg^{-1} min^{-1} in the transgenic rice plants. The enzyme activities are expressed as fold increase relative to those in nontransformants (0.015 μmol mg^{-1} min^{-1}). The PEPC activity of maize leaves was 0.63 μmol mg^{-1} min^{-1}, which was equivalent to 42 fold higher than that of nontransformants data not.

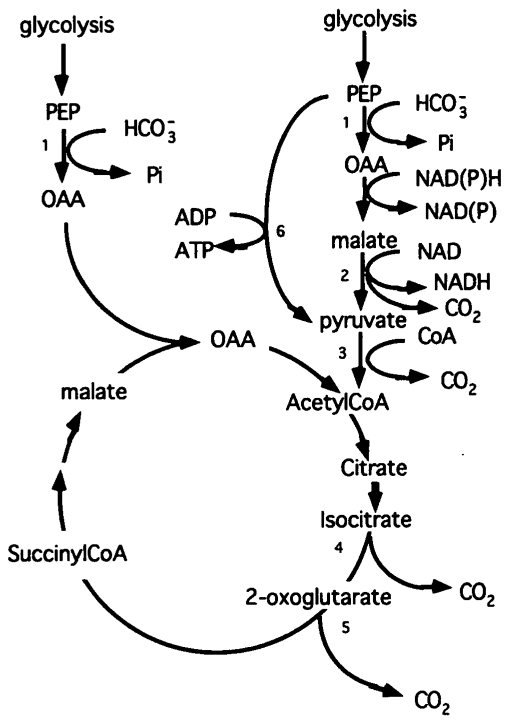

Fig. 2. Simplified metabolic scheme for CO_2 release via citric acid cycle accompanied by assimilation of HCO_3^- in transgenic rice plant. For each HCO_3^- incorporation, a maximum of 4 CO_2 are released (at reaction of 2-5 in the figure). To clarify, some of cofactors involved in enzymatic reactions were omitted. The numbers indicates the enzymes involved in the metabolic step : 1, PEPC; 2, NAD malic enzyme; 3, pyruvate dehydrogenase; 4, NAD isocitrate dehydrogenase; 5, 2-oxoglutarate dehydrogenase; 6, pyruvate kinase.

the activity of PEPC was even higher than that in maize leaves, $^{13}CO_2$ was more discriminated than in nontransformants, implying that some CO_2 was fixed twice by Rubisco. We have assumed that this CO_2 might be provided mainly via respiration (Fig. 2). An enhanced PEPC activity would increase the production of malate with consumption of triose phosphate generated via photosynthetic carbon reduction cycle, and the malate is decarboxylated by mitochondrial NAD malic enzyme. Pyruvate formed by this reaction is completely oxidized in the citric acid cycle. Under this assumption, for each HCO_3^- fixed by PEPC, a maximum of four CO_2 are released. Only one HCO_3^- fixed by PEPC is released directly in the form of CO_2 via NAD malic enzyme, whereas the other three CO_2 molecules are derived from glycolytically produced PEP. Thus, as rates of PEP carboxylation are increased, more CO_2 is released than HCO_3^- fixed. In potato, the respiration rate was increased as PEPC activity incresed (Gehlen et al., 1996; Hausler et al., 1999). Similarly, leaves of rice transformants of T3 generation with 20-fold more PEPC activity increased their respiration rate by up to 1.5 fold relative to non-transformed leaves (Agarie et al., 2001). In transformants, Rubisco would refix this CO_2 in the mesophyll, and this may increase the Δ values more than in the nontransformants. This assumption is supported by the theory developed by Farquhar et al., (1982) and Hubick et al., (1986) showing that Δ is positively correlated with the ratio of intercellular (pi) to atmospheric (pa) partial pressure of CO_2 for C_3 plants. An increased respiration rate in the transformants might lead to an increase in the intercellular partial pressure of CO_2 in the leaf of the transformants. The increased Δ may reflect the increased pi/pa over the experimental period.

In conclusion, overexpression of C_4 PEPC in a C_3 leaf led to a dramatic change in carbon isotope discrimination. This indicates that introduction of a single gene could alter carbon metabolism in C_3 plants. This suggests that overexpression of the C_4 PEPC leads to an increase in the overall CO_2 (HCO_3^-) concentration in the whole leaf cells as well as in the vicinity of the active site of Rubisco. If the high level expression of other C_4 key enzymes could be induced in the PEPC transformants, a C_4-like cycle might be operated in the transgenic rice plants.

References

Agarie, S. et al. 2001. Plant Science in press.
Farquhar, G.D. et al. 1982. Aust J. Plant Physiol. 9 : 121-137.
Farquhar, G.D. et al. 1989 Annu. Rev. Plant Physiol. Plant Mol. Biol. 40 : 503-537.
Gehlen, J. et al. 1996. Plant Mol. Biol. 32 : 831-848.
Kogami, H. et al. 1994. Transgenic Res. 3 : 287-296.
Hausler, R.E. et al. 1999. J. Exp. Bot. 50 : 1231-1242.
Hubick, K.T. et al. 1986 Aust. J. Plant Physiol.13 : 803-816.
Hudspeth, R.L. et al. 1991. Plant Physiol. 98 : 458-464.
Ku, et al. 1999. Nature Biotech. 17 : 76-80.
Park, R. et al. 1960. Geochim. Cosmochim. Acta 21 : 110-126.
Sasaki, H. et al. 1996. Plant Cell Physiol. 37 : 1161-1166.
Slack, C.R. et al. 1967. Biochem. J. 103 : 660-665.