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Developmental changes of plant affecting primary photosynthate distribution in rice leaves

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Abstract

Developmental changes of plant in the regulation of photosynthate distribution of leaves were studied in hydroponically cultivated rice by the $^{14}CO_2$ tracer technique and analysis of the activity of the regulatory enzymes, sucrose phosphate synthase (SPS), phospho*enol*pyruvate carboxylase (PEPC), and pyruvate kinase (PK). The distribution of primary photosynthates into sugars, amino acids, organic acids, sugar phosphates, proteins, and polysaccharides was determined by column chromatography. The relative primary photosynthate distribution to the sugar phosphate fraction was significantly larger in the 5th leaf than in the 6th one. Correspondingly, the V_{max} of PEPC was significantly higher in the 5th than in the 6th leaf, while no significant differences between leaves were detected in the other enzymes. As a consequence, the ratio of the V_{max} of SPS and PEPC was lower in the 5th than in the 6th leaf. As the 5th leaf develops before panicle initiation in rice, it predominantly supports vegetative growth, while the 6th leaf develops after panicle initiation and thus contributes mainly to reproductive growth. We conclude that the physiological properties of each leaf are regulated developmentally. When the 6th leaf became fully expanded (corresponding to the panicle initiation stage of plant), the distribution pattern of ^{14}C was transiently changed in the 5th leaf, indicating that individual organs that are mainly involved in vegetative development are affected to some extent by the whole-plant-level physiological transformation that occurs at the transition from the vegetative to the reproductive stage.

Additional key words: $^{14}CO_2$; leaf position; *Oryza sativa*; phospho*enol*pyruvate carboxylase; primary photosynthate; pyruvate kinase; sucrose phosphate synthase.

Introduction

Rice plants change their physiological status dramatically when proceeding from the vegetative growth stage to the flowering and maturation stage (Osaki *et al.* 1988, 1991). In particular, nitrogenous compounds (proteins) are vigorously synthesized during the phase of vegetative growth, while saccharides (starch) are predominantly produced during maturation time. Nakamura *et al.* (1997) demonstrated that the distribution of photosynthetically assimilated $^{14}CO_2$ differed between the vegetative and flowering stages; the proportion of ^{14}C incorporated into sugars and starch was higher in the flowering period. On the other hand, different leaves have different physiological roles; for instance, older leaves from the base of the rice stem tend to translocate saccharides to roots while almost all of the photoassimilates from the flag leaf are transferred to the panicle (Tanaka 1961). Generally, there is a strong linkage between leaf physiological status and the developmental stage of the rice plant. Leaves 1 to 5 are formed in the seed and are photosynthetically active during the vegetative growth stage. Leaves 6 to 9 are formed at the vegetative growth stage but function as photoassimilate sources during the flowering stage, while leaves at positions 10 to 12 are formed at the flowering stage and produce photoassimilates during the maturation

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Abbreviations: BSA – bovine serum albumin; DTE – dithioerythritol; DTT – dithiothreitol; EDTA – ethylenediamine tetra-acetic acid; F6P – fructose-6-phosphate; G6P – glucose-6-phosphate; LDH – lactate dehydrogenase; MDH – malate dehydrogenase; PEP – phospho*enol*pyruvate; PEPC – phospho*enol*pyruvate carboxylase; PK – pyruvate kinase; PMSF – phenylmethyl sulfonyl fluoride; PVPP – polyvinylpyrrolidone; SPS – sucrose phosphate synthase; UDP-Glc – uridine-5'-diphosphoglucose.
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stage.

Mineral nutrient contents change with ontogenetic leaf development. The ratio between the contents of nitrogen (N) and phosphorus (P) in leaves changes greatly during the transition from the vegetative to the flowering and maturation stage irrespective of leaf position in rice and maize, but not in the dicotyledonous crops soybean, adzuki bean, potato, and sugar beet (Osaki 1995). Thus, the physiological status of gramineous leaves appears to change in accordance with the demands of carbon (C) and N at different growth stages.

Many enzymes involved in the metabolic regulation of photosynthate distribution such as phospho*enol*-pyruvate carboxylase (PEPC) regulate C flow from triose phosphates to the pools of organic acids and amino acids (Champigny and Foyer 1992, Quy *et al*. 1991, Quy and Champigny 1992, Foyer *et al.* 1994). Sucrose phosphate synthase (SPS) regulates the carbon flow for the synthesis of sucrose (Worrel *et al.* 1991, Signora *et al*. 1998, Murchie *et al*. 1999). The expression of pyruvate kinase (PK) was decreased by enhanced nitrate contents, but increased when glutamic acid contents were depleted (Sheible *et al*. 1997), indicating that the regulation by PK of the carbon flow from glycolysis to the TCA cycle is itself controlled by the N content in the cell.

SPS and PEPC are regulated in a similar manner. Introduction of exogenous SPS into plants leads to an increase of the ratio of sucrose to starch in rice (Takahashi *et al*. 2000), and to an enhanced dry matter accumulation in the reproductive organs of tomato (Laporte *et al*. 1997, 2001) and *Arabidopsis* (Signora *et al*. 1998). Thus, changes in the activity of SPS will directly affect carbon flow not only in leaves but also on the whole plant level. While SPS activity is down-regulated by nitrate (Quy *et al*. 1991, Quy and Champigny 1992, Foyer *et al*. 1994), PEPC is activated by PEPC protein kinase (Duff and Chollet 1995, Li *et al*. 1996) which is more active under high nitrate conditions (Quy and Champigny 1992, Mahn *et al*. 1993, Duff and Chollet 1995), probably due to increased gene expression (Mahn *et al.* 1993).

In our previous paper (Shinano *et al*. 2006), we have analyzed the effects of N nutrition on the contents of SPS and PEPC. We found that the leaf N status contributed little to the regulation of the distribution of ^{14}C to primary photosynthate compounds, while the developmental status of plant on the leaf appeared to be a more decisive determinant in the regulation of the distribution of photoassimilates into the C or N pools. As sugar phosphates are the major intermediates in the biosynthesis of photosynthesis-derived compounds, they need to be analyzed in detail to allow an identification of regulatory anabolic steps. In the present study, we evaluated whether the demand from sinks can alter the physiological status of leaves of rice, through the analysis of ^{14}C distribution patterns and SPS, PK, and PEPC enzyme activities. We especially focused on differences between leaves active during the vegetative growth stage and during the flowering stage.

Materials and methods

Plants: Rice (*Oryza sativa* L. cv. Michikogane) seeds were sown on 13th June and transplanted to 56 000 cm^3 vats in batches of 28 on 2nd July when the plants had reached their 3rd leaf stage; they were then kept in a greenhouse of Hokkaido University. The average day length in July is 15 h and average day/night temperature in the greenhouse was 24/18 °C. The vats contained a nutrient solution made up of [μg cm^{-3}] N (NH_4NO_3) 30, P (NaH_2PO_4) 2, K (K_2SO_4 : KCl = 1 : 1) 30, Ca ($CaCl_2$) 50, Mg ($MgSO_4$) 20, and trace elements including Fe, Mn, B, Zn, Cu, and Mo at concentrations of 2.0, 0.5, 0.5, 0.2, 0.01, and 0.005, respectively. The pH of the solution was adjusted to 5.0 daily at 07:00 and 16:00, and the solution was renewed every week. Plants were sampled on 13th July (5th leaf stage), 16th July (6th leaf stage), 21st July (7th leaf stage), and 26th July.

Before sampling, whole plants were allowed to assimilate $^{14}CO_2$. Each plant was covered with a clear polyethylene bag (10 000 cm^3) filled with air. $^{14}CO_2$ was liberated by mixing 1 cm^3 0.18 mM $NaHCO_3$, 0.74 MBq $NaH^{14}CO_3$, and 1 cm^3 of 30 % $HClO_4$ within the bag. The ambient CO_2 concentration was around 0.036 % while the content of CO_2 generated was about 5×10^{-7} %; thus no significant CO_2 enrichment occurred in this experiment. After 5 min exposure to $^{14}CO_2$ under natural irradiation [in excess of 1 000 μmol(photon) m^{-2} s^{-1}; the experiments were performed between 10:00 and 12:00], leaves were removed, frozen in liquid nitrogen, and then lyophilized and stored at –80 °C for subsequent analysis. For enzyme analysis, leaves were collected between 10:00 and 12:00, and samples of leaf tissue (0.2 g) were frozen in liquid nitrogen and stored at –80 °C for analysis. The remaining leaf tissue was dried in an air-forced oven for 72 h at 80 °C for mineral analysis.

Nitrogen content: Dried samples were digested with sulfuric acid and hydrogen peroxide (Mizuno and Minami 1980). Total N was measured by the semi-micro Kjeldahl method (Hind 1993).

Extraction and fractionation of photosynthates: Two-step leaf extractions were carried out using methanol, chloroform, and water (12 : 5 : 3, v/v/v) in the first step, and 0.2 mM formic acid in 20 % ethanol in the second. The combined water-soluble supernatant was separated into organic acid, amino acid, sugar, and phosphate ester fractions by ion-exchange chromatography using *SP Sephadex* C-25 (cation resin, *Amersham Biosciences*,

Piscataway, NJ, USA) and *QAE Sephadex A-25* (anion resin, *Amersham Biosciences*) as described by Redgwell (1980). To measure the amounts of amino acids in proteins, the insoluble fraction (residue after extraction) was hydrolyzed and purified through *SP Sephadex C-25* according to the method of Shinano *et al.* (1994). The residual fraction (= total ^{14}C – soluble ^{14}C – protein ^{14}C) was assumed to represent the starch fraction because assimilated ^{14}C in residual fraction becomes incorporated into starch within minutes after exposure. The radioactivity of each fraction was determined by liquid scintillation counting (*Aloka*, Liquid Scintillation Counter *LSC-5100*, Tokyo, Japan).

Fractionation of sugar phosphates: The sugar phosphate fraction was further fractionated by paper chromatography using *Whatman 31 ETCHR* paper (Whatman, Kent, UK). The media were 2-propanol : ammonia : water, 6 : 2 : 1 v/v/v (first dimension) and *n*-propyl acetate : formic acid (90 %) : water, 11 : 5 : 3 v/v/v (second dimension). The distribution pattern of radioactivity on the chromatogram was visualized using an imaging plate *(BAS-MP, Fuji Film*, Tokyo, Japan) and a Bio Imaging Analyzer (*BAS-1000, Fuji Film*).

Enzyme extraction: For the analysis of SPS and PEPC activities, frozen leaf samples (0.2 g) were homogenized in 0.8 cm^3 extraction buffer containing 100 mM Tris-HCl pH 7.5, 5 % (m/v) glycerol, 5 % (m/v) ethylene glycol, 5 mM sodium phosphate, 50 mM NaF, 10 mM EDTA, 14 mM 2-mercaptoethanol, 2 mM benzamidine-HCl, 1 mM PMSF, 10 μM leupeptin, 16 μM chymostatin, 1 μM microcystin-LR, and 5 % (m/v) PVPP. After centrifugation at 17 000×*g* for 5 min at 4 °C, the supernatant was collected as a crude extract. For the determination of SPS activity, 100 mm^3 of the supernatant was desalted on a *Sephadex G-25* column equilibrated with 50 mM MOPS-NaOH pH 7.5, 10 mM $MgCl_2$, 1 mM EDTA, 25 mM DTT, 10 % glycerol, and 0.1 % (m/v) PMSF. Then the eluate was frozen in liquid nitrogen and stored at –80 °C until analysis. For the determination of the activated state of PEPC, 150 mm^3 of saturated $(NH_4)_2SO_4$ solution were mixed well with 100 mm^3 of the supernatant, placed on the ice for 10 min, frozen in liquid nitrogen, and stored at –80 °C until analysis. For the quantification of maximum activities of PEPC and PK, 100 mm^3 of the untreated supernatant were frozen in liquid nitrogen and stored at –80 °C for analysis.

Enzyme activities: V_{max} and V_{limit} of SPS were determined based on Huber *et al.* (1989). After centrifugation of the desalted supernatant described above at 17 000×*g* for 5 min at 4 °C, 25 mm^3 of the supernatant were added to 45 mm^3 reaction buffer (V_{max}: 50 mM MOPS-NaOH (pH 7.5), 15 mM $MgCl_2$, 2.5 mM DTT, 10 mM F6P, 40 mM G6P, 10 mM UDP-Glc; and V_{limit}: 50 mM MOPS-NaOH (pH 7.5), 15 mM $MgCl_2$, 2.5 mM DTT, 3 mM F6P, 12 mM G6P, 10 mM UDP-Glc, 10 mM P_i); the mixture was incubated at 37 °C for 15 min. After the reaction had been stopped by the addition of 30 % KOH, the solution was kept on a boiling water-bath for 10 min. After cooling on ice, 1 cm^3 of a 0.14 % (m/v) anthron/H_2SO_4 solution was added and the reaction was allowed to proceed for 20 min at 40 °C. A sample to which anthron/H_2SO_4 had been applied before the incubation at 37 °C served as a control. The absorbance at 620 nm was monitored by spectrophotometry (*Shimadzu UV-1600*, Tokyo, Japan); one unit of activity was defined as the amount of protein which produced 1 mol sucrose 6-phosphate in 1 min.

To measure the maximum activities of PK and PEPC, the untreated supernatant of the centrifuged crude extract was used. PK V_{max} activity was determined according to Plaxton (1998). Thirty mm^3 of the supernatant were added to 928 mm^3 of a solution containing 50 mM HEPES-NaOH (pH 6.9), 10 mM $MgCl_2$, 2 mM DTE, 0.15 mM NADH, 0.2 mg cm^{-3} BSA, 2 units of LDH, and 20 mm^3 100 mM ADP. The reaction was started by adding 20 mm^3 of 100 mM PEP, and the absorbance at 340 nm was followed at 30 °C for 15 min. For PEPC V_{max}, 25 mm^3 of the supernatant were combined with 908 mm^3 of a solution containing 100 mM HEPES-NaOH (pH 7.3), 10 mM $MgCl_2$, 1 mM $NaHCO_3$, 0.2 mM NADH, 2 units of MDH, and 25 mm^3 200 mM G6P. The reaction was started by adding 40 mm^3 100 mM PEP, and the absorbance at 340 nm was monitored at 30 °C for 15 min.

To determine the PEPC activation state, the crude extract combined with $(NH_4)_2SO_4$ was centrifuged at 17 000×*g* for 5 min at 4 °C, and the supernatant was discarded. The pellet was re-suspended with 100 mm^3 buffer [50 mM HEPES-NaOH (pH 7.5), 5 % (v/v) glycerol, 5 mM $MgCl_2$, 1 mM EDTA, 14 mM 2-mercaptoetanol, 1 mM PMSF, 16 μM chymostatin] and centrifuged at 17 000×*g* for 10 min at 4 °C. Twenty mm^3 of the supernatant were added to 914 mm^3 of a solution containing 100 mM HEPES-NaOH (pH 7.3), 10 mM $MgCl_2$, 1 mM $NaHCO_3$, 0.2 mM NADH, 2 units of MDH, and the PEPC activation state was assayed by comparing the activity of PEPC in the presence or absence of 40 mM malate (20 mm^3). The reaction was started by adding 40 mm^3 50 mM PEP, and monitored by following the absorbance at 340 nm for 15 min.

Total soluble protein was measured by the method of Bradford (1976), using BSA as a standard.

Results

Growth and N nutrition: Panicle initiation started when the 6th leaf was fully expanded, and thus we assume that photosynthesis in the 5th leaf contributed mainly to vegetative growth while the 6th and younger leaves predominantly fuelled reproductive growth. Leaf N contents were similar in all leaves and growth stages examined (Fig. 1).

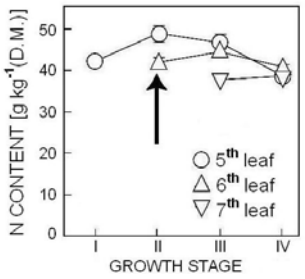

Fig. 1. Nitrogen contents of leaves at different growth stages. I, II, III, and IV indicate growth stages at which the 5th, 6th, 7th, and 8th leaves, respectively, were fully expanded. *The arrow* indicates the panicle initiation stage. Means with SE (n = 3).

Primary photosynthate distribution: A general linear model (GLM) analysis was performed to find differences in the distribution patterns of different groups of photosynthesis products in leaves at different positions. There were significant differences between the 5th leaf on one hand and the 6th and 7th leaf on the other in the sugar fraction, between the 5th and 6th leaf in the sugar phosphate fraction, and between the 7th and the other leaves in the organic acid fraction. From the 5th leaf fully expanded stage to the 7th leaf fully expanded stage, the distribution of assimilated ^{14}C to the sugar phosphate fraction was transiently increased by a factor of two in the 5th leaf (Fig. 2).

In sugar phosphate fraction, the major compounds labelled with ^{14}C were 3-PGA followed by hexose-6-phosphate and F-1,6-BP (Fig. 3). The distribution ratio to each sugar phosphate compound was higher in the 5th leaf than in the 6th one, and it was highest when the 6th leaf had fully expanded; thereafter it gradually decreased.

SPS activity: Neither SPS V_{max} and SPS V_{limit} nor the ratio of the two parameters were significantly different between leaves (Fig. 4). Similarly, no significant difference between leaves was detected by GLM analysis.

PEPC activity: PEPC V_{max} generally increased during the time of observation but dropped again in the 5th leaf after the 7th leaf was fully expanded (Fig. 5). The PEPC inhibition ratio appeared constant over time (Fig. 5). Nevertheless, a significant difference in PEPC inhibition rate between the 5th and 6th leaves was found by GLM analysis.

PK activity: PK V_{max} was more or less constant over time and similar in all leaves examined (Fig. 5). GLM analysis did not reveal significant differences between leaves.

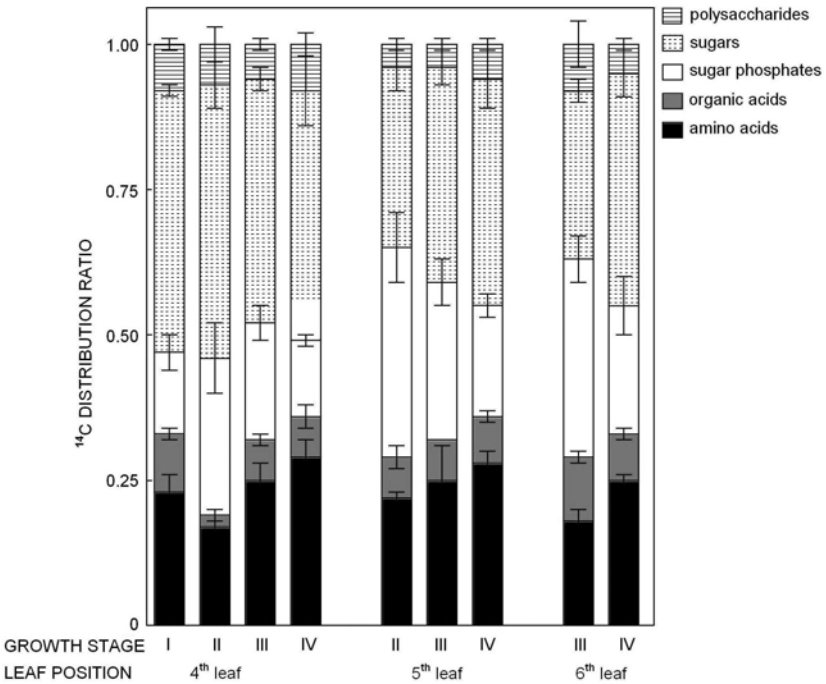

Fig. 2. Primary photosynthate distribution in different leaves at different growth stages. I, II, III, and IV indicate growth stages at which the 5th, 6th, 7th, and 8th leaf, respectively, were fully expanded. Means with SE (n = 3). There were significant differences between the 5th and other leaves in the sugar fraction, between the 5th and 6th leaf in the sugar phosphate fraction, and between the 7th and other leaves in the organic acid fraction. Differences were analysed using the General Linear Model followed by Bonferoni's post hoc test with p<0.05. As protein fraction was negligible in all the case, data were not shown in the figure.

Discussion

The C flow from photosynthesis has been proposed to be directed by SPS into the C pool (sugars and starch; Worrel *et al*. 1991, Signora *et al*. 1998, Murchie *et al*. 1999) or, alternatively, by PEPC into the N pool (amino acids and proteins; Champigny and Foyer 1992, Foyer *et al*. 1994, Huber *et al*. 1994, Stitt 1999). We have corroborated this idea by an analysis of photosynthate distribution in rice leaves at different levels of N nutrition (Shinano *et al*. 2006). In rice, the physiological function of each leaf changes during the plant development; leaves formed early in the phase of vegetative growth of plant support the development of younger leaves and the root system, while leaves formed towards the end of the vegetative phase of plant tend to translocate almost all of their assimilated C to the reproductive organs (Tanaka 1961, Osaki *et al*. 1988, 1991). We hypothesized that the regulation of C flow by the relative activities of SPS and PEPC might correspond to these differences in leaf function. Therefore, we analyzed ^{14}C distribution in leaves after $^{14}CO_2$ assimilation, and the activities of SPS and PEPC in different leaves and growth stages of plant. Plant growth stage-dependent effects on the distribution pattern of primary photosynthates were observed only transiently at the panicle initiation stage (6th leaf fully expanded) in the 5th leaf, suggesting that the physiological characteristics of a leaf are determined by leaf position and therefore probably are genetically rather than environmentally regulated. When mineral nutrient status of leaves was evaluated (Osaki 1995), it was demonstrated that N contents were regulated in dependence on the developmental stage rather than on environmental factors. The regulation by developmental stage seems to occur in the primary photosynthate distribution also, because the GLM analysis revealed a significant difference in the distribution of primary photosynthates to the sugar phosphate pool between the 5th and 6th leaves. As panicles are initiated after the development of the 5th leaf, the 5th leaf is classified as mainly contributing to vegetative growth, whereas the 6th and all younger leaves support reproductive growth.

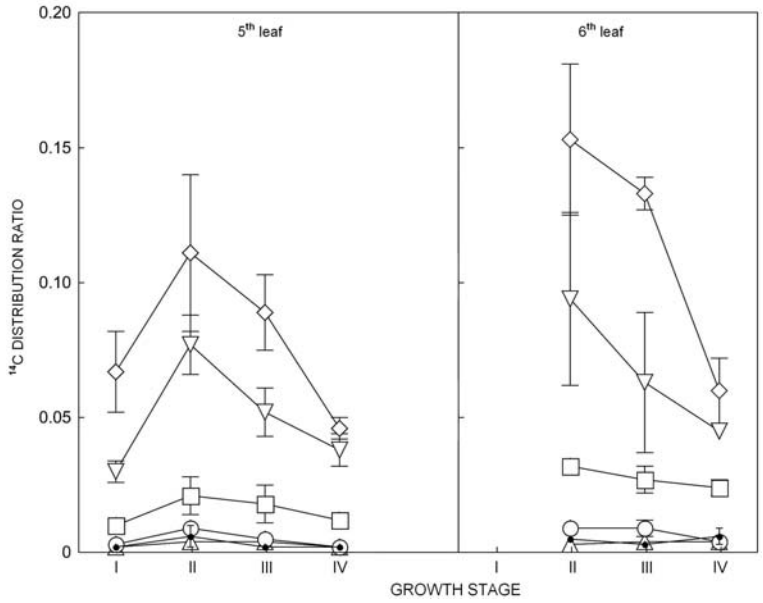

Fig. 3. Distribution of primary photosynthates of the sugar phosphate fraction in the 5th (*left*) and 6th leaf (*right*) at different growth stages. I, II, III, and IV indicate growth stages at which the 5th, 6th, 7th, and 8th leaf, respectively, were fully expanded. Means with SE (n = 3). □ – fructose-1,6-bisphosphate, Δ – UDP glucose, ∇ – hexose-6-phosphate, ◊ – 3-phosphoglyceric acid, ○ – phospho*enol*pyruvate, ● – glucose-3-phosphate.

In contrast to PEPC, the activities of SPS and PK appeared constant irrespective of leaf number and growth stage of plant. As PEPC V_{max} of the 5th leaf was high especially during the early vegetative growth stage, we plotted the ratio of the V_{max} of SPS and that of PEPC against the growth stages (Fig. 5). The relative activity of SPS was lower in the 5th than in the 6th and 7th leaves before the 7th leaf was fully expanded. Higher proportions of ^{14}C are directed into the N metabolism-related pool (amino acids and organic acids) during the vegetative growth stage, whereas after this stage, fluxes into the C metabolism-related pool (sugars and starch) become dominant (Nakamura *et al*. 1997). While the enzyme activities explain the differences in the distribution patterns to some degree, the function of C metabolites (hexose, sucrose, *etc*.) in sensing and signalling the nutritional status of the plant (Halford and Paul 2003) must also be taken into account. Sucrose may control the initiation of the reproductive stage; exogenous sucrose was able to restore the normal phenotype in the late floral transition mutant of *Arabidopsis* (Ohto *et al.* 2001), and over-expression of SPS caused early flowering (Laporte *et al*. 1997, Baxter *et al*. 2003).

If the physiological characteristics of a leaf are determined mostly by the source-sink balance of the whole plant, the C metabolism (which we have characterized

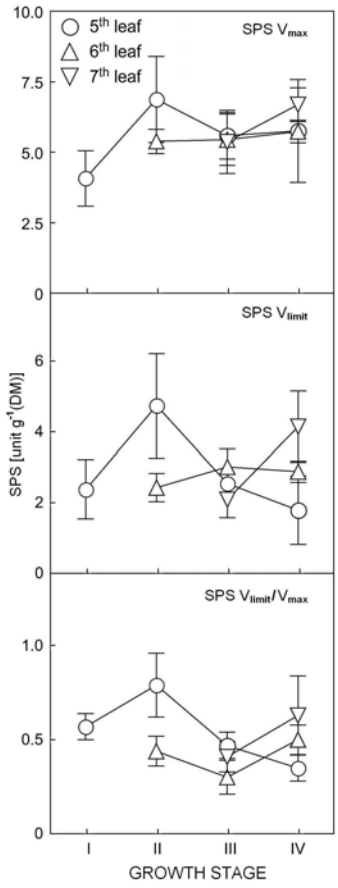

Fig. 4. Sucrose phosphate synthase (SPS) activity and the ratio of V_{limit} and V_{max} at different growth stages. I, II, III, and IV indicate growth stages at which the 5th, 6th, 7th, and 8th leaf, respectively, were fully expanded. Means with SE (n = 3).

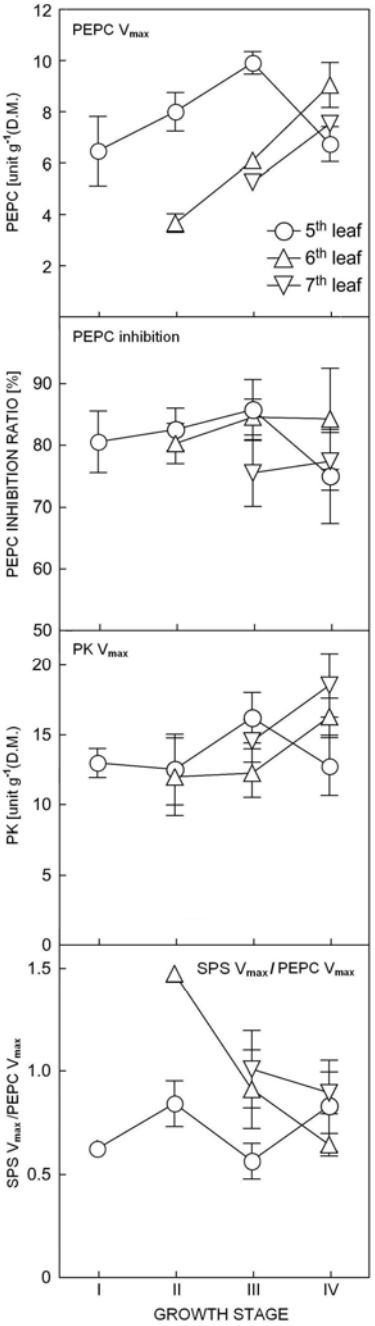

Fig. 5. Phospho*enol*pyruvate carboxylase (PEPC) activity, the ratio of PEPC V_{limit} and V_{max} (PEPC inhibition ratio), phospho-*enol*pyruvate kinase (PK V_{max}) activity, and ratio of SPS V_{max} and PEPC V_{max} at different growth stages. I, II, III, and IV indicate growth stages at which the 5th, 6th, 7th, and 8th leaf, respectively, were fully expanded. Means with SE (n = 3). There were significant differences between the 5th and 6th leaves in the ratio of SPS V_{max} and PEPC V_{max} as revealed by General Linear Model analysis followed by Bonferoni's post hoc test with p<0.05.

by the distribution of primary photosynthates; Fig. 2) would be expected to change at the transition from vegetative growth to the flowering stage. However, we merely observed a transient change in the primary photosynthate distribution pattern in the 5th leaf at the time when the 6th leaf had fully expanded; this effect could be explained by an increase in sugar phosphates (especially 3-phosphoglycerate, 3PGA). As 3PGA is the initial product of the ribulose-1,5-bisphosphate carboxylase reaction, it appeared that a retardation of photosynthate utilization occurred during this transient stage. The relative activities of SPS and PEPC corresponded to the distribution ratio to sugar phosphates (Figs. 2 and 5). Though we had not quantified the sugar phosphate fraction in our previous report (Shinano *et al*. 2006), it seems that the relative distribution of C to sugar phosphates is an indicator of the rate of transformation of primary photosynthates into derived compounds. In the 5th leaf, the ratio of the V_{max} values of SPS and PEPC was lower than in the 6th and 7th leaves. This would be expected to decrease the C flow to sucrose synthesis, but the proportion of ^{14}C distribution to the sugar fraction did not corroborate the idea. In our previous study (Shinano *et al*. 2006), we found a positive relationship between the ratio of the V_{max} of SPS and PEPC on one hand and the proportion of ^{14}C distribution to sugars on the other, but we had not analyzed the sugar phosphate fraction. Sugar phosphates are metabolic intermediates and form substrates for SPS and PEPC (Champigny and Foyer 1992). The relatively higher proportion of primary photo-

synthate distribution to this fraction seems to indicate a retardation of carbon flow to sugar and/or organic acid synthesis through SPS or PEPC, or possibly a re-distribution of PEP into the chloroplasts (Shinano *et al*. 2005). In rice, C distribution between chloroplasts and cytoplasm depends on the plant P status (Shinano *et al*. 2006), and the ratio of N to P contents is greatly modified during the transition from vegetative to reproductive growth of plant (Osaki 1995). This suggests that P metabolism is another important factor that regulates C flow in rice, which will be addressed in future studies.

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"Photosynthetica" in its eighth five-year period

In this period volumes 40 to 44 were published, each contained 640 pages and indexes. Part of issue 3 of volume 43 (2005) contained papers presented at the minisymposium "Chlorophyll Fluorescence in Life of a Man and Plants" which was dedicated to Pavel Šiffel, an untimely deceased Czech biophysicist and researcher of chlorophyll.

The Editorial Board lost in the period 2002−2006 one of its members, serving on the board of Photosynthetica from the very beginning, André Pirson (for obituary see Photosynthetica **42**: 321-322, 2004). One member resigned on his service on the Board because of retirement (H.K. Lichtenthaler), another one because of lack of time (J.-M. Ducruet). Eight new members started to serve on the Board in this period: T. Janda (from vol. 41), B. Gielen, A. Post, A. Telfer, and Z. Tuba (from vol. 42), C. Buschmann and L. Gratani (from vol. 43), and P. Mohanty (from vol. 44). Starting with volume 44 Photosynthetica has also an Executive Editor, Ivana Štětinová. Full texts of Photosynthetica papers can be found regularly on internet.

In the volumes 40−44, Photosynthetica has published 16 reviews on various topics of photosynthesis, 337 original full-length papers, 81 brief communications, 89 book reviews, 5 parts of the "Bibliography of Reviews and Methodological Papers on Photosynthesis" containing 3289 references, 1 obituary, 1 letter to the Editor, 1 report on a photosynthesis meeting, and 1 editorial. Papers in the fields of physiology and ecology of photosynthesis prevailed also in this period among the presented papers; many papers dealt with or used the methods measuring fluorescence induction of chlorophyll *a*. Due to the present perfect databases on the net, the bibliographic section of Photosynthetica ends its service with part 90 (presented in vol. 44).

The total number of published reviews, original full-length papers, and brief communications was 434. The members of the Editorial Board rejected in the respective period 126 manuscripts, which was 31.3 % of all articles sent to the journal. Very rarely a paper was published without modifying it by the authors according to the comments of members of our Editorial Board.

According to the address given by its first author, the reviews, original papers, and brief communications originated from Angola (2), Australia (1), Austria (2), Belgium (2), Bolivia (1), Brazil (10), Bulgaria (6), Canada (5), China (129), Colombia (3), Czech Republic (40), Egypt (4), Estonia (1), France (2), Germany (10), Greece (6), Hungary (6), India (55), Iran (1), Israel (2), Italy (22), Japan (18), Korea (8), México (1), the Netherlands (3), Pakistan (5), Poland (10), Portugal (5), Romania (1), Russia (4), Singapore (1), Slovakia (2), Slovenia (3), South Africa (2), Spain (15), Sweden (2), Taiwan (6), Turkey (2), the U.K. (1), Ukraine (3), the U.S.A. (24), and Venezuela (8). Hence our authors worked in 42 countries of all five continents.

Zdeněk ŠESTÁK
Editor-in-Chief