Proc. Indian natn. Sci. Acad. **B53** No. 5 & 6 pp. 493–498

Regulation of Photosynthetic Efficiency in C_3 Plants

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Three selected C_3 species viz: *Coriandrum sativum* L., *Phaseolus radiatus* L. and *Pisum sativum* L., differing widely in net photosynthetic rates, were compared for the kinetic characteristics of ribulose 1,5-bisphosphate (RuBP) carboxylase and the capacity for sucrose synthesis. The activity of RuBP carboxylase was closely correlated with the rates of net photosynthesis. The leaves of *C. sativum* which exhibited extremely high rates of photosynthesis (39.5 mg CO_2 $dm^{-2}hr^{-1}$) also showed higher RuBP carboxylase activity (365μmol mg $chl^{-1}hr^{-1}$) due to higher affinity of the latter towards CO_2 [lower Km (CO_2)]. The rate of ^{14}C-incorporation into sucrose which varied from species to species was in fact related to sucrose phosphate synthase activities. Qunatitative difference in the levels of sucrose, dihydroxyacetone phosphate (DHAP), fructose 1,6-bisphosphate and fructose 2,6-bisphosphate during steady state photosynthesis suggested that partitioning of photosynthetically fixed carbon influences the maximal rates of photosynthesis in C_3 plants.

Key Words: Photosynthesis, Sucrose synthesis, C_3 plants, *Coriandrum sativum*

Introduction

Identification of constraints limiting the photosynthetic performance of higher plants could be used for prognosis of plant productivity. Sufficient information is available to elucidate the photosynthetic efficiency in C_4 plants. Earlier, we compared several C_3 plant species selected for high and low photosynthetic rates on a leaf area basis in combination with biomass yields and found that net photosynthetic rates were well correlated to the dry matter production (Ramachandra Reddy & Das 1986). Further, the kinetic characteristics of RuBP carboxylase were more critical in determining the rates of CO_2 assimilation in C_3 plants.

A number of mechanisms have recently been proposed for the regulation of photosynthetic efficiency in C_3 plants. These regulation processes are thought to coordinate sucrose synthesis with rates of net assimilation of CO_2 (Stitt et al. 1984, Ramachandra Reddy & Rama Das 1987). Recently, fructose-2, 6-bisphosphate (F-2, 6-P_2), an effector metabolite, has been identified in leaves, which could integrate carbon metabolism as a regulatory link between chloroplasts and cytosol (Cseke et al. 1982, Huber 1986). An inverse relationship between photosynthetic rate and leaf F-2, 6-P_2 concentration has recently been reported (Stitt et al. 1984, Ramachandra Reddy & Rama Das 1987). The present study was initiated to identify any obvious differences in certain biochemical characteristics of photosynthesis which might explain the differences in net CO_2 assimilation patterns of certain selected C_3 plants.

Materials and Methods

Plants of *Coriandrum sativum* L., *Phaseolus radiatus* L. and *Pisum sativum* L. were grown under natural photoperiod (day/night temperature 33/25 ± 1°C, average mid-day, photosynthetic photon flux density, 1700–1900μmol photons $m^{-2}s^{-1}$). Young and fully expanded leaves of 5-week old plants were used for experimentation. Radiation measurements were made with Li-Cor LI 170s light meter equipped with a quantum sensor (Lambda Instruments, Lincoln, NE).

Gas exchange measurements were made on an infra-red gas analyser (Analytical Development Co. Ltd., England) using open gas system. A plexiglass cuvette (4 × 4 × 0.5 cm) was used as the photosynthetic chamber. Air containing 334μl CO_2 l^{-1} was passed through the chamber at a flow rate of 30 l hr^{-1}. Illumination was provided by halide flood lamp behind a water screen. Photorespiratory rates were measured followed by the technique of CO_2 evolution into CO_2 free air (Moss 1966). CO_2 compensation point was determined using the gas analyser in a differential mode. Leaf conductance of water vapour was measured with a Li-Cor steady state diffusion porometer and

conductance of CO_2 was calculated as 0.625 times leaf conductance of water vapour.

$^{14}CO_2$ incorporation into metabolites was determined by enclosing fully expanded leaves of similar age in a plexiglass chamber, sealed with foam rubber. $NaH^{14}CO_3$ (16.5 KBq μmol^{-1}) was introduced into the chamber and $^{14}CO_2$ was liberated by injecting 32% lactic acid at time zero. The leaves were allowed to photosynthesize for the appropriate time at a light intensity of 1500μmol $m^{-2}s^{-1}$ and at the end of fixation, the leaves were plunged in liquid nitrogen. The ^{14}C incorporated into individual photosynthetic intermediate compounds was determined according to Benson et al. (1950).

Estimation of Metabolites: Two grams of leaf material was frozen in liquid nitrogen and homogenized with 20 mM HEPES-NaOH buffer (*p*H 8.2) which contained 5 mM EDTA, 65% (v/v) methanol and 25% (v/v) chloroform. The extract was centrifuged at 2000 g for 5 min. The supernatant was dried at 45°C and redissolved in 1 ml distilled water.

Fru 2, 6-P_2 was assayed in a Hitachi-557 double beam-double wavelength spectrophotometer as described by Van Schaftingen et al. (1982). Sucrose contents were measured according to Jones et al. (1977). Triose phosphate and fructose 1,6-bisphosphate contents were estimated according to Wirtz et al. (1980).

Extraction and Assay of Enzymes: Enzyme extraction was done at 0–4°C. 10 g of leaves were homogenized in a blendor in 35 ml of 50 mM HEPES-NaOH (*p*H 7.5) which contained 5 mM $MgCl_2$, 5 mM DTT, 1 mM EDTA, 0.5% BSA and 1% polyvinylpyrollidone-40. The extract was centrifuged at 1000 g for 2 min to remove the debris. The supernatant was recentrifuged at 30,000 g for 20 min. The resulting supernatant was passed through Sephadex G-25 which was pre-equilibrated with the extraction buffer. All the enzymes (except SP synthase, RuBP carboxylase) were assayed by following the changes in absorbance at 340 nm in Hitachi-557 spectrophotometer at 30°C. Chlorophyll content was determined in 80% acetone (Arnon 1949).

RuBP carboxylase activity was assayed at 30°C by the incorporation of $^{14}CO_2$ into acid stable products. The assay mixture contained (3 ml) : 100 mM Bicine-NaOH (*p*H 8.0), 5 mM DTT, 20 mM $MgCl_2$, 10 mM $NaH^{14}CO_3$ (16.5 KBq μmol^{-1}), 0.5 mM RuBP and the enzyme protein. Reaction was started by the addition of 20μl of the activated enzyme and stopped by adding 0.3 ml of 2 M acetic acid. The vial contents were evaporated to dryness under an electric lamp. The acid-stable radioactivity was determined in Beckman LS 1800 liquid scintillation system. RuBP oxygenase was assayed according to Lorimer et al. (1977).

Phosphoglucose isomerase was assayed in a reaction mixture which included 50 mM tris-HCl buffer, *p*H 8.0, 0.2 mM, NADP, 5 mM $MgCl_2$, 3 mM FBP, 5 units glucose-6-phosphate dehydrogenase and the enzyme.

Phosphoglucomutase was assayed using the reaction mixture which contained 50 mM tris-HCl buffer, *p*H 8.0, 0.2 mM NADP, 5 mM $MgCl_2$, 5 units glucose-6-phosphate dehydrogenase, 5 mM glucose-1-phosphate and the enzyme.

UDPG pyrophosphorylase was followed by a coupled reaction. The reaction mixture (1.0 ml) contained 50 mM HEPES-NaOH, *p*H 7.5, 5 mM UDPG, 5 mM $MgCl_2$, 0.3 mM NADP, 2 mM sodium pyrophosphate, 3 units glucose-6-phosphate dehydrogenase, 2 units of phosphoglucomutase and the enzyme preparation.

Sucrose phosphate synthase was assayed at 30°C by measuring the production of UDP (Huber 1981). The reaction mixture (2 ml) contained 50 mM HEPES-NaOH (*p*H 7.5), 8 mM UDPG, 8 mM F-6-P, 10 mM $MgCl_2$ and the enzyme. The reaction was terminated by adding 0.1 ml 1 N NaOH. The tubes kept in boiling water for 10 min to remove the unreacted F-6-P. After cooling 0.5 ml of 0.1% (v/v) resorcinol in 95% ethyl alcohol and 1 ml of 30% HCl were added. The tubes were incubated at 30°C for 10 min. The reaction tubes were cooled and the absorbancy at 520 nm was recorded.

Fructose-6-P, 2-kinase (F-6-P-2K) was assayed as described by Soll et al. (1985).

Cytosolic fructose-1, 6-bisphosphatase activities were assayed according to the method of Zimmerman et al. (1978). Protein contents in the extracts used for enzyme assays were determined by the method of Lowry et al. (1951).

Results

The leaves of *C. sativum* exhibited exceptionally high rates of photosynthesis (39.5 mg CO_2 $dm^{-2}hr^{-1}$) compared to commonly reported values for other C_3 plants. However the rate of photorespiration and CO_2 compensation point were comparable to those of C_3 plants (table 1). Stomatal conductance at saturating photosynthetic photon flux density was also significantly high (412 mmol $m^{-2}s^{-1}$) with the leaves of *C. sativum*. The leaf anatomy *C. sativum* was typical of a C_3 plant. The extremely high net photosynthesis rates in *C. sativum* were well related to significantly high activities of RuBP carboxylase and to higher ratio of RuBPC/RuBPO (8.78) as shown in (table 2). High activities of RuBP carboxylase in *C. sativum* were further related to significantly low Km (CO_2) value (7.2μM) which was obtained with purified leaf extracts. The Vmax values of the enzymes from *C. sativum* was also extremely high (table 2). $^{14}CO_2$

Table 1 *Photosynthetic characteristics in the leaves of three C_3 plants**

Experiment	*Coriandrum sativum*	*Phaseolus radiatus*	*Pisum sativum*
CO_2 assimilation (mg $dm^{-2}hr^{-1}$)	39.5 ± 2.6	23.5 ± 1.3	24.8 ± 1.7
CO_2 compensation point ($\mu l\ l^{-1}$ CO_2)	45.6 ± 3.5	50.9 ± 4.1	53.8 ± 4.7
Photorespiration (mg $dm^{-2}hr^{-1}$)	5.3 ± 0.08	6.2 ± 0.15	5.9 ± 0.09
Stomatal conductance at saturated PPFD (m mol $m^{-1}s^{-1}$)	412.0 ±18.6	380.5 ±20.1	372.9 ±19.7

*Values are the mean of 5 experiments ± SE

Table 2 *Activities of carboxylase, oxygenase and sucrose phosphate synthase in leaf extracts and phtosynthetic end product formation in three C_3 plants*

Experiments	*Coriandrum sativum*	*Phaseolus radiatus*	*Pisum sativum*
RuBP carboxylase†	365.09 ±11.32	291.19 ±8.02	282.01 ±10.51
Km (CO_2) of RuBP carboxylase (μM)	7.25 ±0.23	10.18 ±0.35	9.37 ±0.38
Vmax (μmol mg $prot^{-1}min^{-1}$)	2.01 ±0.09	0.98 ±0.06	1.02 ±0.08
RuBP oxygenase†	41.56 ±4.02	42.80 ±3.10	43.33 ±4.15
RuBPC/RuBPO ratio	8.78 ±0.49	6.96 ±0.52	6.50 ±0.48
Sucrose phosphate synthase†	15.82 ±1.06	8.61 ±0.92	9.24 ±0.98
Photosynthetic end product formation (After 10 min)			
Sucrose (μmol mg chl^{-1})	2.20 ±0.09	1.61 ±0.06	1.52 ±0.07
Sucrose (% of total ^{14}C)	85.20 ±5.60	68.17 ±4.31	69.01 ±5.32
Starch (% of total ^{14}C)	12.00 ±0.91	13.12 ±0.80	12.82 ±0.89
Sucrose/Starch ratio	7.10 ±0.52	5.19 ±0.43	5.73 ±0.50

*Values are the mean of 5 experiments ± SE
†(μmol mg $chl^{-1}hr^{-1}$)

fixation by the leaves of *C. sativum* under steady state conditions indicated an unusual accumulation of label in sucrose (up to 85%) after 10 min of $^{14}CO_2$ fixation which amounted to 2.2μmol mg chl^{-1} (table 2), thus resulting high ratio of sucrose: starch, compared to other C_3 plants.

During rapid photosynthetic state (11.00 hr) the levels of triose phosphate (DHAP) and sucrose, fructose 1,6-phosphate and fructose 2,6-bisphosphate were estimated in the leaf extracts (table 3, figure 1). Significantly higher concentration of DHAP was noticed in the leaves of *C. sativum* than was found in the leaves of *P. radiatus* and *P. sativum* under conditions of maximum photosynthesis. The contents of fructose 1,6-bisphosphate was also high in the leaves of *C. sativum* under steady state photoysnthetic conditions while there was no significant differences in the levels of fructose 2, 6-bisphosphate among the three plants examined (table 3). However under limiting light intensities when the rates of photosynthesis varied, an inverse relationship was observed between the concentrations of DHAP and sucrose and fructose 2,6-P_2 over a range of conditions between rapid and decreased rates of photosynthesis (figure 1). Data on the activities of enzymes related to sucrose synthesis in the leaf extracts were depicted in table 4. The activities were estimated at 11.0 hr when maximum photosynthesis was noticed by the leaves. The levels of fructose 1,6-bisphosphatase (FBPase) and sucrose phosphate synthase (SP synthase) were higher in the leaf extracts of *C. sativum* than those in *P. radiatus* and *P. sativum* (table 4). The kinetic properties of FBPase, a key enzyme in sucrose synthesis, were determined using the leaf extracts. The Km (FBP) was extremely low for the enzyme in *C. sativum* (6.8μM).

Table 3 *Net photosynthetic rates and the contents of triose phosphate, fructose 1,6-bisphosphate and fructose 2,6-bisphosphate in the leaves of three C_3 plants at saturating light intensity**

Plant	Photo-synthesis (mg CO_2 $dm^{-2}hr^{-1}$)	DHAP (nmol mg chl^{-1})	F-1,6-P_2 (nmol mg chl^{-1})	F-2,6-P_2 (nmol mg chl^{-1})
Coriandrum sativum	39.52 ± 4.3	88.81 ± 6.3	96.39 ± 8.5	72.10 ± 6.8
Phaseolus radiatus	23.35 ± 2.5	60.18 ± 5.3	70.32 ± 6.7	80.34 ± 8.2
Pisum sativum	25.19 ± 3.1	58.36 ± 4.8	75.18 ± 7.5	78.32 ± 8.0

*Values are the mean of 5 experiments ± SE

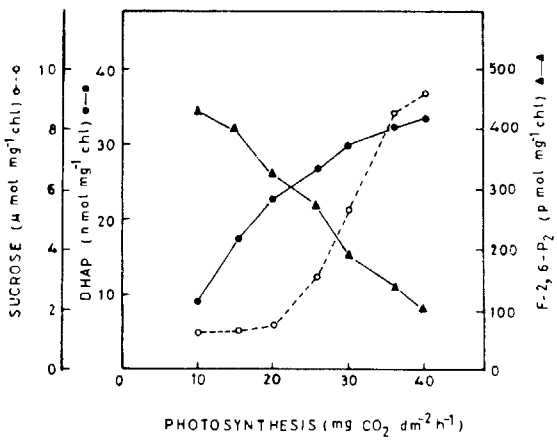

Figure 1 Relationship between photosynthesis and the levels of sucrose, DHAP and fructose 2,6-bisphosphate in the leaves of *Coriandrum sativum*. The varied rates of photosynthesis were obtained under limited light intensities

Table 4 *Activities of fructose 1,6-bisphosphatase, UDPG-pyrophosphorylase, sucrose phosphate synthase, phosphoglucose isomerase and phosphoglucomutase in the leaf extracts of three C_3 plants**

Enzyme	*Coriandrum sativum*	*Phaseolus radiatus*	*Pisum sativum*
	(μmol mg chl^{-1}hr^{-1})		
Fructose 1,6-bisphosphatase	128.3 ±10.8	98.5 ±11.2	96.3 ±9.6
UDPG-pyrophosphorylase	81.8 ±9.3	71.5 ±6.9	73.8 ±8.2
Sucrose phosphate synthase	15.8 ±1.8	8.6 ±1.2	9.2 ±1.3
Phosphoglucose isomerase	216.2 ±16.5	188.9 ±18.6	195.6 ±18.8
Phosphoglucomutase	203.5 ±20.6	190.8 ±18.5	185.6 ±19.3

*Values are the mean of 5 experiments ± SE

Table *Kinetic properties of cytosolic fructose 1,6-bisphosphatase activity in the leaf extracts of three C_3 plants**

Plant	Km (FBP) (μM)	Vmax (μmol mg prot^{-1}min^{-1})
Coriandrum sativum	6.8	0.53
Phaseolus radiatus	10.55	0.32
Pisum sativum	11.4	0.35

*Values are the mean of 3 experiments

Correspondingly the Vmax was higher in *C. sativum* than those from *P. radiatus* and *P. sativum*. The effect of DHAP on the activities of F-6-P-2K in *C. sativum* was determined (figure 2). A 2-fold decrease in the activity was observed in presence of DHAP (0.5 mM).

Discussion

The rates of net photosynthesis in the leaves of coriander (39.5 mg CO_2 dm^{-2}hr^{-1}) are among the highest ever reported for a wide range of native agricultural C_3 species and are comparable with those reported for certain C_4 plants (Raghavendra & Das 1976). Data on photosynthetic characteristics of the three C_3 plants showed a reasonably good agreement between the RuBP carboxylase activity and the net photosynthetic rates. Kinetic characteristics of RuBP carboxylase have been compared for several C_3 plants in relation to photosynthetic rates which revealed certain consistent patterns (Ramachandra Reddy & Das 1986). The variations in Km (CO_2) among C_3 and C_4 plants and the differences in specific activity of RuBP carboxylase have been shown in recent surveys (Zima & Sestak 1977, Yeoh et al. 1981, Seeman et al. 1984 and Castrillo 1985). The Km (CO_2) observed with the purified enzyme from the leaf extracts of *C. sativum* is sufficient to account for the *in vivo* rates of photosynthesis. The variations in photosynthetic performance among C_3 plants is partially controlled by the kinetic efficiency of the carboxylating system. Any attempt to alter the kinetic properties of the enzymes may alter the specific activity leading to improved photosynthetic productivity at least in C_3 plants.

It has been reported that mechanisms that control sucrose synthesis will modulate the reductive fixation of CO_2 in the chloroplasts (Stitt 1986). A restriction of sucrose synthesis would lead to lower photosynthetic rates because of the accumulation of surplus photosynthate. However partitioning of photosynthate will depend upon the relative amounts of different enzymes in the leaf. For example in the present study we provide an evidence for the variation in the kinetic properties of FBPase, a key enzyme in sucrose synthesis, among the C_3 plants. FBPase from coriander

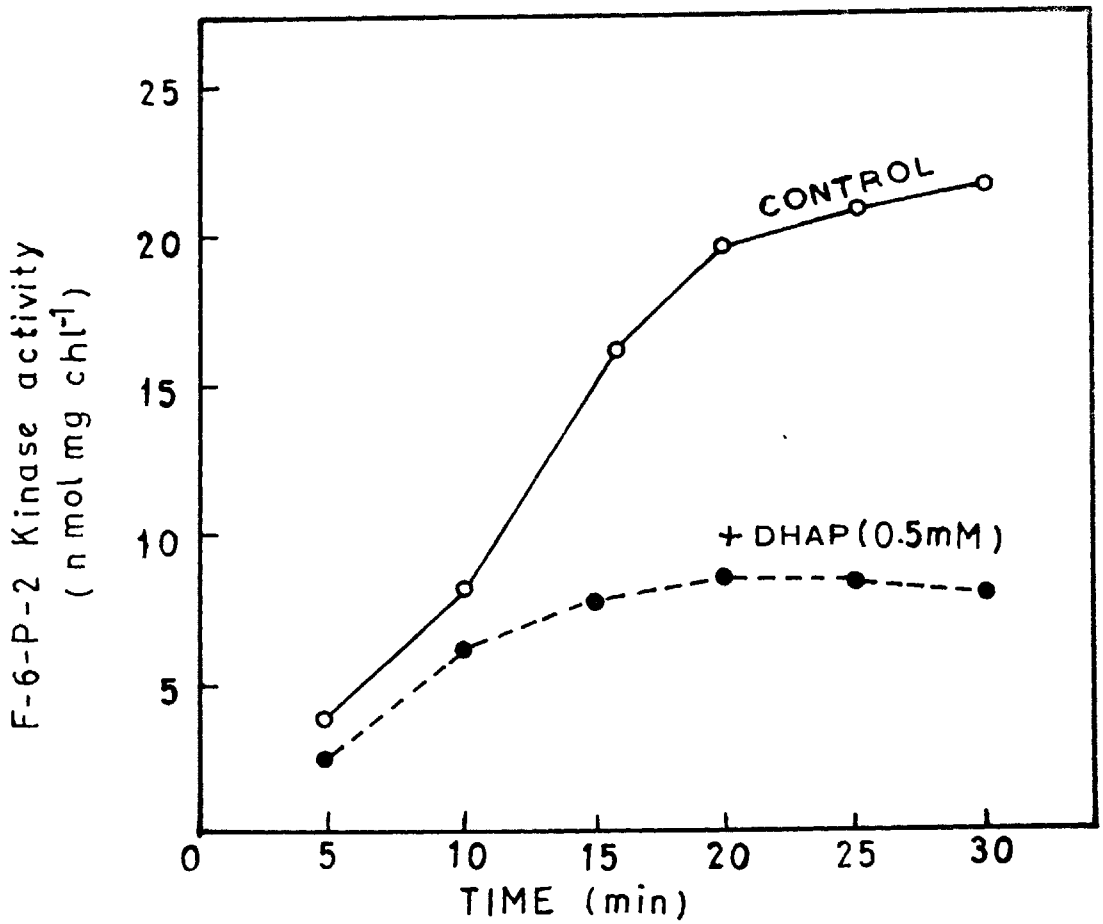

Figure 2 Effect of DHAP (0.5 mM) on the time course of the activity of fructose-6-phosphate-2-kinase in the leaves of *Coriandrum sativum*

leaves requires 2-fold higher concentrations of the substrate than the enzymes from *Phaseolus* or *Pisum*. The activities of sucrose-P synthesizing enzyme, SP synthase were also high in the leaves of *C. sativum* which were well correlated with the rate of sucrose synthesis in the leaves. It is thus evident that variations in FBPase levels are responsible for differences in the accumulation of sucrose. However the rate of sucrose synthesis is dependent upon the levels of triose-P in the stroma. Adequate levels of triose-P are necessary both to maintain the turnover of the Calvin cycle and to maintain the rates of sucrose synthesis for the regeneration of Pi. Our study indicates that maintenance of higher concentrations of DHAP is essential for maintaining rapid sucrose synthesis and for maintenance of high rates of photosynthesis. Our data also confirm that there are large variations in the concentrations of chloroplast metabolites regulating the sucrose metabolism.

Variations in sucrose synthesis in the present study may not be due to variations in the levels of F-2, 6-P_2. There was not much variation in the contents of F-2,6-P_2 among the C_3 plants during rapid photosynthesis. Eventhough accumulation of F-2, 6-P_2, the effector metabolite, is known to suppress sucrose synthesis, the present study indicates that high levels of DHAP will alleviate the F-2, 6-P_2 limited sucrose synthesis by inhibiting the synthesis of F-6-P-2K. To test the validity of this concept we examined the effect of DHAP on the activity of F-6-P-2K (figure 2). The results indicate a 50% inhibition of F-6-P-2K activity in presence of 0.5 mM DHAP. This ensures that maintenance of high DHAP levels are needed to derive an effective mechanism of sucrose synthesis thus favouring a fine control system to maintain rapid photosynthesis. It is thus believed that altering the target enzymes such as FBPase and SP synthase in a more specific way may lead to an essential regulatory control over photosynthetic performance in C_3 plants.

Acknowledgement

This research was supported by a grant from United States Department of Agriculture (FG-IN-576, *IN-SEA-171*).

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