PHOTOSYNTHETICA 34 (3): 419-426, 1997

Carbon allocation and partitioning in *Vigna radiata* (L.) Wilczek as affected by additional carbon gain

Aruna SHARMA and U.K. SENGUPTA

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110 012, India

Abstract

Carbon allocation to the source leaf, export and partitioning to the sink were studied in mungbean supplied by additional carbon from the source leaves subjected to high CO_2 concentrations (600 and 900 $cm^3\ m^{-3}$) in three metabolic and functional source-sink combinations. The plants were pruned to a source-path-sink system. With CO_2 enrichment there was an appreciable increase in net photosynthetic CO_2 uptake in earlier formed and physiologically younger leaves. Most of the carbon fixed as a result of enrichment was translocated out of the source leaf within one diurnal cycle. The carbon remaining in the source leaf was unchanged. Partitioning of extra carbon into starch or sugar depended upon the amount of extra carbon synthesized. The unloading of the extra carbon into sinks depended on whether it was used for growth or stored. Under increased carbon content, the leaf as a sink was able to reorganize its metabolic reactions more rapidly to maintain the required gradient for unloading than the pod acting as the sink.

Additional key words: CO_2 enrichment; leaf age; mungbean; net photosynthetic rate; ^{14}C photosynthates; sink; starch; sugars.

Introduction

Conversion of atmospheric CO_2 to organic compounds increases the amount of carbon available for transport to places of various processes such as growth and storage. The amount of CO_2 fixed gives the upper limit of the carbon available for use in the source organ, and for export and partitioning among various sinks. CO_2 enrichment raises the amount of available carbon by enhancing CO_2 fixation (Bishop and Whittingham 1968, Aoki and Yabuki 1977). As a result, the saccharide pool of the source leaf increases, *e.g.*, the starch content (Madsen 1968, Cave *et*

Received 14 November 1995, *accepted* 23 July 1997.

Acknowledgements: This research has been financed partly by a grant from the U.S. Department of Agriculture, Office of the International Cooperation and Development, authorised by Public Law 480.

al. 1981). However, there are conflicting reports as to the share of the extra carbon fixed that is exported to growing and storage organs or stored as starch in leaves. Huber *et al.* (1984) did not find any increase in carbon export from leaves under CO_2 enrichment while Ho (1977) reported increased rate of CO_2 fixation as well as of transport in tomato. The present study deals with the allocation of extra carbon synthesized as a result of CO_2 enrichment to the source leaf, and with its transport to the sink in metabolically and functionally distinct source-sink combinations in mung bean within one diurnal cycle.

Materials and methods

Plants of *Vigna radiata* (L.) Wilczek cv. PS-16 were grown in earthen pots (40 cm diameter) filled with farmyard manure and sandy loam soil (1:1) under natural environment. Plants were pruned to a source-path-sink system 24 h prior to high CO_2 feeding at following three stages of plant growth:

Stage I: Plants were 25 d-old, had a pair of cotyledonary leaves, first and second mature leaves, and third developing leaf. 12 d-old 2nd leaf was used as source leaf, and developing 3rd leaf (3-4 d-old) as the sink.

Stage II: Plants were 44-45 d-old, and pod filling had started. 12 d-old 5th leaf and 4 d-old pod were used as source and sink, respectively. First flower of the 1st flush of flowering was tagged to ascertain pod age.

Stage III: Plants were 55-57 d-old. 18 d-old 5th leaf and almost mature pod (10 d-old) were taken as source and sink, respectively.

CO_2 treatment: Source leaf in each case was subjected to 350, 600, and 900 $cm^3\ m^{-3}$ CO_2 (treatments c_{350}, c_{600}, and c_{900}, respectively). A steady-state open system for feeding CO_2 enriched air was used. CO_2 air mixture of desired concentration was generated by mixing pure CO_2 gas (obtained from commercial source) with ambient air in a 0.04 m^3 glass container. Approximately 10 and 22 cm^3 of pure CO_2 was added to the jar to raise its concentration to 600 and 900 $cm^3\ m^{-3}$, respectively. The mixture was thoroughly shaken, and the final CO_2 concentration was adjusted by diluting the enriched air by ambient air using a vacuum pump. One of the outlets from the jar was attached to an infra-red gas analyser (*ADC*, England) that continuously displayed the CO_2 concentration in the jar during dilution. The mixture so generated maintained the CO_2 concentration within 5 $cm^3\ m^{-3}$ of the target concentration.

The source leaf was enclosed in a plexiglass chamber, and CO_2 was fed with flow rate of 400 $cm^3\ m^{-3}$ for 6 h under halogen quartz lamp yielding a photon flux density of 1000 $\mu mol\ m^{-2}\ s^{-1}$. Leaf temperature was maintained at 30 °C. Net photosynthetic rate (P_N) was determined by the infra-red gas analyser (*ADC*, England) in the absolute mode according to Palta (1983). P_N was corrected for expansion of air at different temperatures. CO_2 depletion was obtained from the differences in the CO_2 concentration of enriched air present in the jar and that of the enriched air passing through the leaf chamber.

Carbon distribution: ^{14}C-labelled CO_2 was fed to the source leaves (for 1 h) after the leaves were exposed to ambient or high CO_2 for a period of 2 h. Labelled CO_2 was generated by adding 1 M HCl to 0.2 cm^3 of $NaH^{14}CO_3$ (92.5 kBq) placed in the jar containing high CO_2 air mixture. The source leaves were then allowed to photosynthesize in ambient or enriched air without tracer for 3 h, and returned to natural environment. After a pulse-chase period of 24 h the plants were separated into source leaf, path, sink organ, and the rest of the pruned shoot. Each plant part was chopped finely, and extracted in 80 % ethanol. The pooled extract in scintillation vials was evaporated to dryness at 60 °C. Samples were bleached in hydrogen peroxide. Radioactivity was measured with a *Philips* scintillation counter. Simultaneously, standards under identical matrix conditions using plant materials were used for self-absorption correction (Chase and Rabinowitz 1967).

Starch and sucrose contents were estimated at the end of feeding with CO_2. Sucrose was extracted in 95 % ethanol, the solution was clarified, and hydrolysed (McCready *et al.* 1950). The resulting reducing sugars were determined by the arsenomolybdate method of Nelson (1944) using the improved copper reagent of Somogyi (1952). The amounts of sucrose were obtained by the differences in values of reducing sugars in the extracts before and after hydrolysis. The dried residue was hydrolysed in a glycerine bath, and starch content was determined by the anthrone method (McCready *et al.* 1950). The starch content was calculated by multiplying glucose values with 0.9 (Pucher *et al.* 1948). The values presented are means from at least four replicates.

Results

P_N: An appreciable increase in CO_2 uptake rate was observed in c_{600} and c_{900} leaves of all ages at various positions (Table 1). Compared with ambient air treatment, second leaf at stage I showed a 2.5-fold increase in c_{600}, and 4.5-fold increase in c_{900}. Fifth leaf of the same physiological age (12 d) showed poorer increase (1.8 and 2.7 times in c_{600} and c_{900}, respectively), and the 18 d-old 5th leaf showed the least response to CO_2 enrichment.

Table 1. Changes in net photosynthetic rate (P_N) [mg(CO_2) m^{-2} s^{-1}] at various CO_2 concentrations (c_{350}, c_{600}, c_{900}) [cm^3 m^{-3}] at three stages of growth in mungbean.

	P_N stage I	stage II	stage III
c_{350}	11.80	9.80	7.80
c_{600}	37.10	22.09	17.73
c_{900}	40.03	29.03	22.10
C.D.	stage 1.92	concentration 1.83	stage × conc. 2.89

Carbon distribution: At c_{350} the 12 d-old 5th leaf exported 54 % of the total label found in the plant as against 32 % exported by the 2nd leaf of the same physiological age, while the 18 d-old leaf at the same position on the main shoot exported 48.4 % of the total plant radioactivity. At stage I, 59.9 and 67.4 % of the total radioactivity was transported out of the 2nd leaf at c_{600} and c_{900}, respectively (Fig. 1). Since the incorporation of label in enriched leaves was higher, it was necessary to examine the actual radioactive counts found in each plant part rather than consider their proportion as the only basis. The amount of label in the CO_2-enriched leaves was nearly equal to that found in c_{350} leaves in all the source-sink combinations. Most of the extra carbon fixed as a result of enrichment was translocated out of the source leaf within one diurnal cycle.

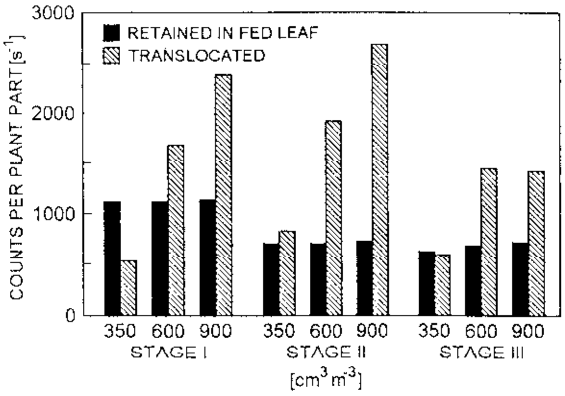

Fig. 1. Translocation of ^{14}C assimilates from the source leaf exposed to high CO_2 at three stages of growth in mungbean.

Values of carbon distribution to the sink (Table 2) revealed that leaf as a sink got 56.7 % of the total radioactivity exported out of the source leaf as against 38 % received by the pod. Near-mature pod was able to unload 56 % of the total radioactive carbon compared to 38 % unloaded into the immature pod. Under c_{600} and c_{900}, the 2nd leaf took 91.0 and 76.2 % of the labelled carbon exported out of the source leaf whilst the amount retained in the rest of the shoot decreased to 8.9 and 23.8 %, respectively. Even though the proportion of ^{14}C-labelled CO_2 in the leaf sink was smaller in c_{900} than in c_{600}, the amount of carbon unloaded was greater because the source leaf in c_{900} had a higher P_N than in c_{600}. At stage II, the developing pods showed a progressive increase in the proportion of labelled assimilates unloaded in c_{350}, c_{600}, and c_{900} treatments. The proportion of the label in the path remained the same while that in the rest of the pruned shoot decreased. At stage III, the near mature pod showed a decrease in the proportion of assimilates unloaded into it in c_{900} though the actual counts in the pod were greater than in the c_{600} treatment.

Starch and non-reducing sugar contents: To ascertain whether the extra carbon synthesized was used immediately for export or was stored as starch, the contents of starch and non-reducing sugars were estimated after exposure to CO_2 enriched air. In all three source leaves in c_{600} the concentration of non-reducing sugars (primarily

Table 2. Allocation of translocated ^{14}C to various sinks at three stages of growth in mungbean and at three CO_2 concentrations (c_{350}, c_{600}, c_{900}) [cm^3 m^{-3}]. Means ± S.E. Figures in parentheses represent the percentage of translocated ^{14}C present in each plant part.

Stage		Total ^{14}C translocated [s^{-1}]	^{14}C in the sink	^{14}C in the path	^{14}C in the rest of shoot
I	c_{350}	524 ± 12	297 ± 16	-	226 ± 36
	c_{600}	1673 ± 56	1523 ± 39	-	1489 ± 21
	c_{900}	2351 ± 144	1791 ± 134	-	593 ± 18
II	c_{350}	833 ± 17	316 ± 12	217 ± 36	292 ± 11
	c_{600}	1907 ± 105	804 ± 27	499 ± 37	443 ± 13
	c_{900}	2673 ± 132	1336 ± 391	719 ± 15	617 ± 13
III	c_{350}	597 ± 18	3490 ± 391	110 ± 13	139 ± 5
	c_{600}	1449 ± 17	696 ± 18	399 ± 8	467 ± 9
	c_{900}	1414 ± 39	747 ± 36	431 ± 6	233 ± 7

sucrose) increased (40 %) but there was little increase in the starch content, while in c_{900} the concentration of non-reducing sugars was comparable to that found in c_{600} but the starch content was appreciably higher than that in c_{600} (Fig. 2).

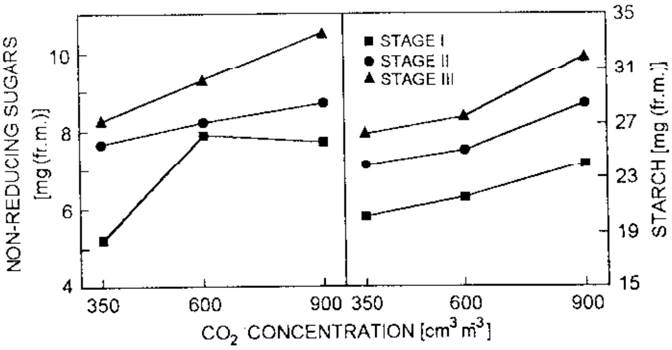

Fig. 2. Starch and total sugar contents of source leaves exposed to high CO_2 at three stages of growth in mungbean.

Discussion

Four-fold increase in photosynthetic rate due to CO_2 enrichment has been reported in several C_3 species (Hesketh *et al.* 1984, Campbell and Young 1986; for review see Saralabai *et al.* 1997, for models see Yamaguchi 1996). Our results showed that physiological age did affect the response of leaf P_N to increased CO_2 concentration. Leaves at different positions on the main stem showed variation even when they were of the same physiological age. Hicklenton and Jolliffe (1980) found in tomato leaves a larger increase in net CO_2 exchange up to 0.1 % CO_2 concentration at LPI-5 than at LPI-10.5. Leaves of the same age but at different positions on the main shoot (12 d-old 2nd and 5th leaves) exported different amounts of radioactive label. This difference may be due to the nature of the sink. On CO_2 enrichment, incorporation of radioactive label was higher due to increased P_N. The amount of radioactivity in CO_2-enriched leaves was nearly equal after 24 h. This indicates that the amount of extra carbon fixed as a result of CO_2 enrichment was translocated out of the source leaf within one diurnal cycle.

To find out whether the extra carbon was exported out immediately as current photosynthate or stored as starch and then mobilised in the dark period, starch and sugar contents were estimated. In general, the concentration of non-reducing sugars increased at c_{600} with little increase in starch content. The c_{900} treatment resulted in larger partitioning of carbon into starch while the non-reducing sugar content was comparable to that in c_{600} leaves. These results show that the pool of saccharides increases up to a point, after which carbon (probably as triose phosphate) gets diverted towards starch synthesis. Changes in sucrose-starch partitioning on CO_2 enrichement have been reported in soybean (Huber *et al.* 1984). Wardlaw (1982) reported that high CO_2 concentration over a period of 6 h resulted in relatively more of the additional leaf photosynthate being partitioned into storage than exported to other organs. Larger sucrose synthesis on enrichment has been reported in tomato (Ho 1977). Our study shows that allocation of extra carbon towards starch synthesis or towards current export depends on the amount of extra carbon fixed. At c_{600}, the export of current photosynthates increased, and then reached saturation. At c_{900}, carbon was probably diverted towards starch synthesis after sucrose synthesis was saturated. However, extra carbon fixed either as sucrose or as starch was exported out of the leaf within one diurnal cycle, and the carbon allocated to leaf remained unchanged.

The distribution of exported carbon reveals that under c_{350}, the growing meristimatic leaf in stage I was a much more efficient sink for assimilates, than the pod as a storage sink at stage II. The rate of import by a sink would be mainly governed by the allocation processes which may be different in different sinks. In a utilization sink like importing leaf, which consumes carbon, the main metabolic activity is respiration; there is therefore a rapid depletion of sucrose in the consumer cells, and the necessary gradient at the point of unloading from the supply system is always maintained. The leaf as sink was able to unload more of the total label exported out of the source leaf than the pod (Table 2). At stage I, the source leaf and the sink leaf did not have petioles, and there were less anatomical barriers present. Pods near maturity were better at unloading than the young immature pods, even though the nature of the sink was the same (storage) because the biochemical reactions governing actual unloading were different at different stages of pod development. Under c_{600}, the proportion of radioactive label as well as the amount of label in the sink increased whereas at c_{900}, though the amount of label was larger, the proportion of label in the sink was either similar or little less than that found for c_{600}. This indicates that biochemical reactions governing the actual unloading can be changed only to a limited extent under increased availability of current photosynthates. The rate of import may be limited more by the sucrose gradient at the point of unloading than by the rate of unloading *per se*. Wyse and Saftner (1982) reported that rate of sucrose uptake, exhibited by excised tap root tissue, was inversely related to the supply of photosynthate during preceding light period. CO_2 enrichment reduced the uptake capacity compared to control. Thus the exposure of leaves to large concentrations of CO_2 results in increased net carbon exchange leading to increased synthesis of saccharides. A greater part of the current photosynthate was exported out of the source leaf at CO_2 enrichment but the amount of carbon allocated to the source leaf remained unchanged. The amount and proportion of the extra carbon going towards starch synthesis was dependent upon the amount of extra carbon synthesized. Even with more available carbon, sinks were not able to unload carbon due to limitations in the rate at which metabolic adjustments in utilization, transformation or compartmentation of metabolites will be made; these are necessary to maintain the gradient required for unloading. This suggests that potential sink strength is genetically determined; but the actual sink strength is determined by factors which affect the rate limiting processes within the sink (Ho 1988). Improving or manipulating the growing condition to obtain maximum capacity would be ways of increasing yield.

References

Aoki, M., Yabuki, K.: Studies on the carbon dioxide enrichment for plant growth. VII. Changes in dry matter production and photosynthetic rate of cucumber during carbon dioxide enrichment. Agr. Meteorol. **18**: 475-485, 1977.

Bishop, P.M., Whittingham, C.P.: The photosynthesis of tomato plants in a carbon dioxide enriched atmosphere. - Photosynthetica **2**: 31-38, 1968.

Campbell, D.E., Young, R.: Short-term CO_2 exchange response to temperature, irradiance, and CO_2 concentration in strawberry. - Photosynth. Res. **8**: 31-40, 1986.

Cave, G., Tolley, L.C., Strain, B.R.: Effect of carbon dioxide enrichment on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. - Physiol. Plant. **51**: 171-174, 1981.

Chase, R.D., Rabinowitz, J.L.: Principles of Radioisotope Methodology. - Bergers Publ. Co., Minneapolis 1967.

Geiger, D.R., Sovonick, S.A.: Effect of temperature, anoxia and other metabolic inhibitors on tranlocation. - In: Zimmermann, M.H., Milburn, J.A. (ed.): Encyclopedia of Plant Physiology. Vol. 1. Pp. 256-286. Springer-Verlag, Berlin - Heidelberg - New York 1975.

Hesketh, J.D., Woolley, J.T., Peters, D.B.: Leaf photosynthetic CO_2 exchange rates in light and CO_2 enriched environments. - Photosynthetica **18**: 536-540, 1984.

Hicklenton, P.R., Jolliffe, P.A.: Alterations in the physiology of CO_2 exchange in tomato plants grown in CO_2-enriched atmospheres. Can. J. Bot. **58**: 2181-2189, 1980.

Ho, L.C.: Effects of CO_2 enrichment on the rates of photosynthesis and translocation of tomato leaves. - Ann. appl. Biol. **87**: 191-200, 1977.

Ho, L.C.: Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. - Annu. Rev. Plant Physiol. Plant mol. Biol. **39**: 355-378, 1988.

Huber, S.C., Rogers, H., Israel, D.W.: Effects of CO_2 enrichment on photosynthesis and photosynthate partitioning in soybean (*Glycine max*) leaves. - Physiol. Plant. **62**: 95-101, 1984.

Madsen, E.: Effect of CO_2-concentration on the accumulation of starch and sugar in tomato leaves. - Physiol. Plant. **21**: 168-175, 1968.

McCready, R.M., Guggloz, J., Silviera, V., Owen, H.S.: Determination of starch and amylose in vegetables. - Anal. Chem. **22**: 1156-1158, 1950.

Nelson, N.: A photometric adaptation of the Somogyi method for determination of glucose. - J. biol. Chem. **20**: 375-380, 1944.

Palta, J.A.: Photosynthesis, transpiration and leaf diffusive conductance of the cassava leaf in response to water stress. - Can. J. Bot. **61**: 373-376, 1983.

Pucher, G.W., Leavenworth, C.S., Vickery, H.B.: Determination of starch in plant tissue. - Anal. Chem. **20**: 850-853, 1948.

Saralabai, V.C., Vivekanandan, M., Suresh Babu, R.: Plant responses to high CO_2 concentration in the atmosphere. - Photosynthetica **33**: 7-37, 1997.

Somogyi, M.: Notes on sugar determination. - J. biol. Chem. **195**: 19-23, 1952.

Wardlaw, I.F.: Assimilate movement of *Lolium* and *Sorghum* leaves. III. Carbon dioxide concentration effects on the metabolism and translocation of photosynthate. - Aust. J. Plant Physiol. **9**: 705-715, 1982.

Wyse, R.E., Saftner, R.A.: Reduction in sink-mobilizing ability following periods of high carbon flux. - Plant Physiol. **69**: 226-228, 1982.

Yamaguchi, J.: An estimate of the photosynthate pool size from the equilibration of $^{14}CO_2$. - Photosynthetica **32**: 87-95, 1996.