Influence of Translocation on Photosynthetic Efficiency of Phaseolus vulgaris L.

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ABSTRACT

Measurements of net photosynthesis show that in Phaseolus vulgaris L., the cultivar Michelite-62 exceeds the cultivar Red Kidney in net CO₂ uptake by 23 to 31%. Data on translocation of pulse label indicate that export of a pulse of photosynthetically assimilated ¹⁴C from the source leaf of either M-62 or Red Kidney follows an exponential pattern and shows an initial rapid phase followed by a second slower phase. The steeper slope for both phases in M-62 suggests its rate of translocation of pulse label is higher than that of Red Kidney. Furthermore, only 33% of the ¹⁴C remains in the leaf of M-62 after 8 hours, while Red Kidney retains up to 60% of the label. Leaf autoradiographs obtained after pulse labeling demonstrate a much faster rate of vein loading in M-62 and are considered evidence for the higher translocation efficiency of M-62. These results provide evidence for a positive correlation between photosynthetic efficiency and translocation efficiency in M-62 and Red Kidney and give support to our hypothesis that translocation is one of the important physiological factors controlling the varietal differences in photosynthetic efficiency in Phaseolus vulgaris.

Differences in photosynthetic efficiency, defined as the net CO₂ uptake in the light, have been demonstrated widely both among and within species (4). In this laboratory Izhar (7) has reported such differences among cultivars of Phaseolus vulgaris L. He showed that the cultivar Michelite-62 (M-62) exceeded four other varieties in net CO₂ exchange by 9 to 35%. In particular, it consistently and significantly exceeded the cultivar R.K. in net CO₂ exchange at all light intensities above the compensation point. Attention has since been focused in this laboratory on the component physiological processes leading to this varietal difference in photosynthetic efficiency among dry beans (9).

Translocation has long been proposed as an internal factor affecting photosynthesis (10). Hofstra and Nelson (5, 6) showed that export of a pulse label of ¹⁴C from the fed area of a leaf in several species was logarithmic with two components: an initial rapid phase followed by a much slower phase. The rapid phase varied from species to species and a comparison of the percentage of ¹⁴C translocated, and the photosynthetic rate previously reported for these species revealed a very close correlation between the rates of these two processes.

Vein loading was first observed by Barrier and Loomis (1) with detached leaves using ³²P. Recently, Leonard and Glenn (8) using autoradiography described in detail the accumulation of ¹³C-assimilates by veins in the blades and their basipetal transport into petioles in detached leaves of R.K. bean. They suggested that, while basipetal flow was dependent upon magnitude of sink activity, vein loading was an independent and continuous process. Geiger and Cataldo (2) suggested that parenchyma cells of the minor veins actively accumulated sugar prior to its entry into the sieve tube, i.e. vein loading preceded translocation.

In the present study, a hypothesis is proposed that in Phaseolus vulgaris the higher photosynthetic rate observed in certain cultivars is at least partially attributable to a higher translocation rate. Two processes: (a) translocation of pulse label and (b) vein loading were monitored using the two cultivars M-62 and R.K. for comparison.

MATERIALS AND METHODS

M-62 and R.K. plants were grown in pots with a mixture of equal parts by volume of peat, soil, and vermiculite in the growth chamber with a 16-hr light period at an intensity of 2000 ft-c (2.95 mw/cm² [400-725 nm]) provided by fluorescent and incandescent bulbs and an 8-hr dark period. Temperatures in the chamber were controlled at 24 C during the day and 18 C during the night, with relative humidity held at 50%. Soluble fertilizers were added every 10 days.

A simplified one source-one sink plant system similar to that described by Geiger and Swanson (3) was used throughout this study. Sixteen to 17 days after planting the plant was decapitated above the first trifoliate leaf, the two side leaflets of which were also removed. The remaining top leaflet thus became the sole source of photosynthates and is hereafter referred to as the source leaf. The bud in the axil of the source leaf was allowed to grow 4 more days after trimming and represented a major sink.

Measurements of Net Photosynthesis. Net photosynthesis was measured with a system consisting of two leaf chambers, an infrared CO₂ analyzer (Beckman Model 215) and a Cary-Tolbert ionization chamber attached to a vibrating reed electrometer (Cary Model 401), all interconnected to form a closed circuit. The CO₂ analyzer and vibrating reed electrometer were each connected to a recorder and respectively monitored ¹³C₀₂ and ¹⁴C₀₂ (Fig. 1). The difference of CO₂ concentration in the system, ΔCO₂, taken during a definite time period, Δt, along with the photosynthesizing leaf area, A, were used in a com-

computerized calculation of net photosynthesis in µmoles CO₂/min·dm² leaf area. The Plexiglas leaf chamber consisted of a top air chamber and a bottom water chamber each fitted with hose connections to distribute airflow through a pump at a rate of 3 1/min and circulating water flow from a Fisher isotherm refrigerated bath. The top chamber had a slot through which the petiole of a leaf could be inserted; and the leaf blade within the chamber was kept horizontal by a grid of nylon line. This air chamber could be closed at the top with a Plexiglas plate and sealed with an O ring and four wing-nuts. Temperature within the air chamber was monitored with a metal thermometer and was controlled at 25 ± 1°C by means of the water chamber. Light intensity was controlled at 2000 ft·c at the leaf level.

Labeling with ¹⁴CO₂. ¹⁴CO₂ was generated prior to labeling through the reaction of Ba⁴CO₃ (Tracer Lab, specific radioactivity 57.6 mc/mole) with 35% perchloric acid and mixed with a known volume of 100% CO₂ in a 2-liter filtering flask. The top of the flask was connected to a separatory funnel containing H₂O, and the sidearm was connected to the closed circuit through a hypodermic needle. At the start of labeling, the separatory funnel was opened and the ¹⁴CO₂ mixture in the flask was forced into the closed system by the dripping water which was adjusted to maintain the CO₂ concentration at 300 µl/l within the system throughout the labeling period.

Measurement of Translocation of Pulse Label. ¹⁴CO₂ of the specific radioactivity 9.76 mc/mole was administered to the source leaf for 5 min. The leaf was then removed from the leaf chamber, and the export of ¹⁴C was determined by continuous measure of the amount of radioactivity remaining in the leaf with a thin window G-M detector (Nuclear Chicago) which was connected to a ratemeter (Nuclear Chicago Model 1620B) and a recorder (Nuclear Chicago Model R1000). The detector tube was placed vertically beneath the abaxial side of the leaf so that the window was entirely covered for the total experimental period of 8 hr. The continuously recorded cpm readings from the ratemeter were converted to per cent of original and expressed as per cent ¹⁴C remaining in the source leaf. The experiment was performed with both varieties and was repeated several times.

Vein Loading. The source leaves of each set of plants (one of each variety), were exposed to ¹⁴CO₂, (specific radioactivity 6.93 mc/mole) for 4 to 7 min. One set of leaves was detached directly following labeling and quickly frozen with powdered Dry Ice. The time between detaching and freezing of the leaves ranged from 7 to 8 min. Another set was removed from the leaf chamber after labeling and left in air in the light for 10 min, then detached and frozen. Frozen leaves were freeze-dried overnight in a lyophilizer. They were then re-equilibrated by placing above water at 45 ± 0.5°C for 45 min to reduce brittleness, mounted, and exposed to Kodak Royal Blue medical X-ray film for 6 days. The experiment was repeated using intermediate time intervals.

RESULTS AND DISCUSSION

Data on the translocation of pulse label indicate that export of a pulse label of ¹⁴C from the source leaf of either M-62 or R.K. follows an exponential pattern. When the percentage of ¹⁴C remaining in the source leaf following pulse labeling is semilogarithmically plotted against time (Fig. 2), the points fall along straight lines for each cultivar which intersect at approximately 70 min. Both show an initial rapid phase of export...
of ¹⁴C followed by a second slower phase. The slope for both phases is steeper in M-62 than in R.K., indicating that M-62 has a faster rate of export of a pulse of photosynthetically assimilated ¹⁴C for up to 5 hr. Thereafter, R.K. maintains the same rate for the remainder of the 8-hr experimental period, while M-62 shows a third phase with a rate similar to that of R.K. After 8 hr the M-62 leaf retained only 38.5% of the ¹⁴C pulse, whereas the R.K. leaf retained 60%.

 Autoradiographs showing vein loading in the source leaves of M-62 and R.K. following labeling with ¹⁴CO₂ for 4 min are presented in Figure 3. Eleven minutes elapsed from the beginning of labeling to freezing of the leaves. The difference between the two varieties is striking: the veins of M-62 are nearly completely loaded, whereas those of R.K. are still clear. Furthermore, in R.K. the petiole is hardly labeled at this time, while in M-62 at least the upper portion of the petiole is slightly labeled. The relatively light regions around the veins in the leaf lamina of M-62 indicate that the label has left the mesophyll cells and moved into the veins, whereas the lamina of R.K. are still heavily labeled. The light portion of the veins at the leaf base might have resulted from the thickness of the petiole which prevented perfect contact between the mounted leaves and the X-ray film during exposure.

 Autoradiographs of leaves which were labeled and then left in air in the light for 10 min for photosynthesis and translocation before being detached and frozen (elapsed time of 22 min) were similar for both varieties. In this case the major and minor veins of both varieties were loaded, and the petioles were lightly labeled. Autoradiographs for time periods between 11 and 22 min were intermediate in labeling pattern.

 Vein loading has been suggested as a means of driving translocation of organic compounds (2). The autoradiographs clearly demonstrate a much faster rate of vein loading in M-62 compared to R.K., which suggests a higher efficiency of translocation in M-62.

 Net photosynthesis for the partially defoliated plants used in the present experiment was 4.78 ± 0.08 and 3.48 ± 0.24 μmoles CO₂/dm² min for M-62 and R.K., respectively, agreeing with previous findings in this laboratory (7, 9).

 In conclusion, results of the present investigation provide evidence for a positive correlation between photosynthetic efficiency and translocation efficiency in M-62 and R.K. and give support to the hypothesis proposed in this study that translocation is one of the important physiological factors which may well be controlling the varietal differences in photosynthetic efficiency in Phaseolus vulgaris.
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