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## ANALYSIS OF CARBON TRANSLOCATION TO ROOT RESPIRATION IN CUCUMBER PLANTS (*Cucumis sativus* L.) BY $^{13}\text{CO}_2$ TRACING

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### ABSTRACT

YOSHIDA S. and EGUCHI H. *Analysis of carbon translocation to root respiration in cucumber plants (*Cucumis sativus* L.) by  $^{13}\text{CO}_2$  tracing.* BIOTRONICS 21, 25-31, 1992. Dynamics of translocation of photosynthetic carbon to roots of cucumber plants (*Cucumis sativus* L.) was analyzed in controlled environments in hydroponics:  $^{13}\text{CO}_2$  was supplied to leaves under lighting, and the  $^{13}\text{CO}_2$  concentration in nutrient solution was measured in an intact plant by using a gas chromatograph-mass spectrometer. In dynamic responses of  $^{13}\text{CO}_2$  release in roots to  $^{13}\text{CO}_2$  supply to leaves, the delay of the time in translocation of newly fixed carbon in leaves to respiratory carbon release in roots was found to be about 2 h in the cucumber plant at the 3 leaf stage.

**Key words:** *Cucumis sativus* L.; cucumber plant; carbon fixation; carbon translocation; root respiration; stable isotope tracer; mass spectrometer.

### INTRODUCTION

The root activities relate to the carbon balance in a plant. In particular, the root respiration is influenced by photosynthesis and translocation of the carbohydrates to roots (2, 9). The translocation of photosynthetic carbon into root medium has been examined by using isotopic tracers; labelled photosynthetic carbon translocated to roots is respired in roots, and labelled  $\text{CO}_2$  appears in the root medium within 1 h after the carbon fixation in leaves in several plant species (3, 4, 6-8, 13, 14). In the previous paper, the effect of lighting to leaves on gas exchange and water uptake in roots has been reported (10).

The present paper deals with dynamic analysis of translocation of photosynthetic carbon to root respiration in cucumber plants grown in an air-tightened hydroponics (11, 12) by using stable isotope tracer of  $^{13}\text{CO}_2$ .

### MATERIAL AND METHODS

#### *Plant materials*

Cucumber plants (*Cucumis sativus* L. cv. Chojitsu-Ochiai) were grown in fully aerated hydroponics at an air temperature of 23°C and a relative humidity

of 70% in a phytotron glass room. The 3 leaf stage plants (21 days old plants) of healthy growth were used for the experiments. Total leaf area and root dry weight were about 400 cm<sup>2</sup> and 0.14 g, respectively.

#### *Instrumentations*

Figure 1 shows the schematic diagram of the system for control and instrumentations in an air-tightened hydroponics. An air-tightened acrylic chamber ( $4 \times 10^{-3} \text{ m}^3$ ) was used for the supply of  $^{12}\text{CO}_2$  and  $^{13}\text{CO}_2$  to shoot environment, in which air temperature and humidity in the chamber were controlled. The circulation rate of air in the chamber was about  $2 \times 10^{-4} \text{ m}^3 \text{ s}^{-1}$ . A pot ( $1 \times 10^{-3} \text{ m}^3$ ) filled with nutrient solution was air-tightened, and the root environment was completely separated from the shoot environment in the chamber. Root temperature in the solution was controlled by a water bath method. This system was installed in a growth cabinet (5) with artificial light (fluorescent lamps; FLR110EH·W/A, Toshiba Corp.).

$^{13}\text{CO}_2$  was supplied to the shoot environment from the source by manipulating the three-way valve. Total  $\text{CO}_2$  concentration in air was controlled with the use of the feedback signal from  $\text{CO}_2$ -meter (CGP-1, Toa Electronics Ltd.). The excess  $\text{CO}_2$  respired in darkness was manually reduced by the  $\text{CO}_2$  absorber of KOH in order to keep almost the same concentration as that in the light.

Gas exchanges were evaluated by measuring concentrations of  $\text{O}_2$  and  $\text{CO}_2$  in air and in nutrient solution with the use of polarographic  $\text{O}_2$ -meter (UD-1, Central Kagaku Co., Ltd.),  $\text{CO}_2$ -meter (CGP-1, Toa Electronics Ltd.), and pH-meter (HM-7E, Toa Electronics Ltd.). Water uptake rate in roots was measured automatically by a potometer, where the decrease in the solution was detected by a float connected to a potentiometer. The respective sensor signals were transmitted to CPU through interfaces.

#### *Mass spectrometric measurement of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$*

The  $^{12}\text{CO}_2$  and  $^{13}\text{CO}_2$  concentrations ( $[^{12}\text{CO}_2]$  and  $[^{13}\text{CO}_2]$ ) in air and in nutrient solution were measured respectively by using gas chromatograph-mass spectrometer equipped with a pretreatment unit (GCMS-QP 1000 EX, Shimadzu Corp.): The pretreatment unit was used for extraction and purification of dissolved  $\text{CO}_2$  in sampled solution.

#### *Experimental procedures*

Air temperature and relative humidity were controlled at 25°C and 50%, respectively, and root temperature was controlled at 25°C. The light intensity was  $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Aerial  $\text{CO}_2$  concentration was controlled at the set point of 330 ppm ( $13.5 \text{ nmol m}^{-3}$  at 25°C). The  $^{13}\text{CO}_2$  was supplied to the shoot environment (the acrylic chamber) at the concentrations of 80 to 94% of total  $\text{CO}_2$  in air. Dissolved  $\text{O}_2$  was enriched by bubbling  $\text{O}_2$  gas into the solution before the experiments. Dissolved  $\text{CO}_2$  was evaluated as a molarity of total inorganic carbon ( $\Sigma\text{CO}_2$ ) composed of  $\text{CO}_2$ ,  $\text{H}_2\text{CO}_3$ ,  $\text{HCO}_3^-$ , and  $\text{CO}_3^{2-}$ , as

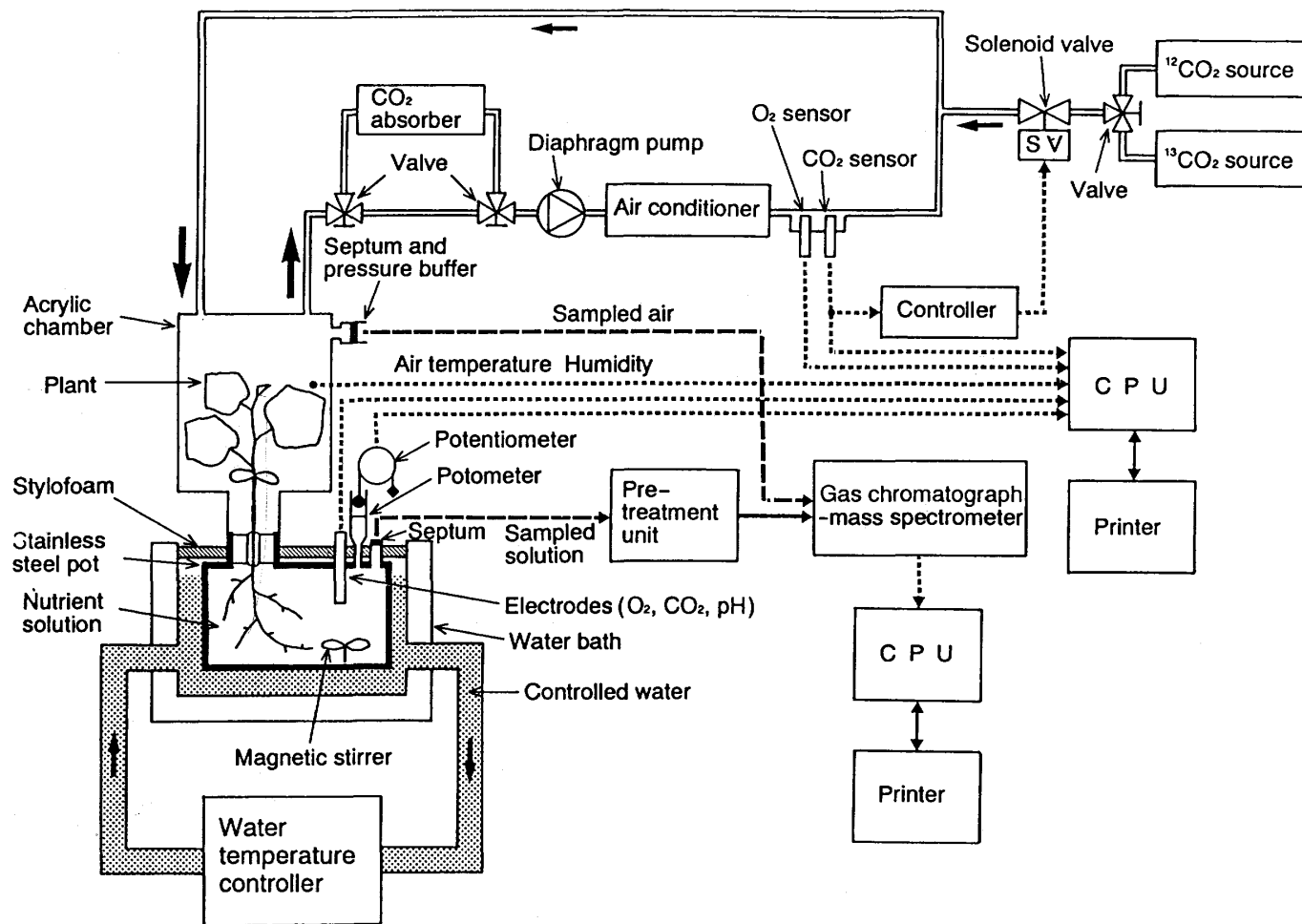


Fig. 1. System diagram of instrumentations for  $^{13}\text{CO}_2$  fixation in leaves and  $^{13}\text{CO}_2$  release in roots in an intact plant in controlled environment: Shoot environment in the acrylic chamber and root environment in the pot were independently air-tightened and controlled respectively, where aerial  $\text{CO}_2$  concentration, air temperature, relative humidity, and root temperature were controlled, and gas exchange and root water uptake were measured on-line. The  $^{13}\text{CO}_2$  in air and nutrient solution was measured by a gas chromatograph-mass spectrometer.

described in the previous paper (12):

$$[\Sigma\text{CO}_2] = [\text{CO}_2 + \text{H}_2\text{CO}_3] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]$$

Air and the nutrient solution were sampled respectively through the rubber septums by using syringes at an interval of 1 h for the mass spectrometric measurement.

## RESULTS AND DISCUSSION

Figure 2 shows respective concentrations of  $\text{O}_2$  and  $\text{CO}_2$  ( $^{12}\text{CO}_2 + ^{13}\text{CO}_2$ ) in air and in nutrient solution under the environments controlled at  $25^\circ\text{C}$  of air and root temperatures and at 50% of relative humidity. The aerial  $\text{O}_2$  gradually increased to  $10 \mu\text{mol m}^{-3}$ , owing to photosynthesis. The aerial  $\text{CO}_2$  concentration (in total) distributed in a region of 11 to  $18 \text{ nmol m}^{-3}$ . Initial concentrations of dissolved  $\text{O}_2$  and  $\text{CO}_2$  in the solution was  $0.5$  and  $0.1 \mu\text{mol m}^{-3}$ , respectively. The dissolved  $\text{O}_2$  linearly decreased to  $0.1 \mu\text{mol m}^{-3}$ , and the dissolved  $\text{CO}_2$  increased to  $0.7 \mu\text{mol m}^{-3}$  in 13 h, by root respiration. Water uptake rate in roots increased to  $1.8 \text{ mg s}^{-1}$  at about 40 min after lighting, and

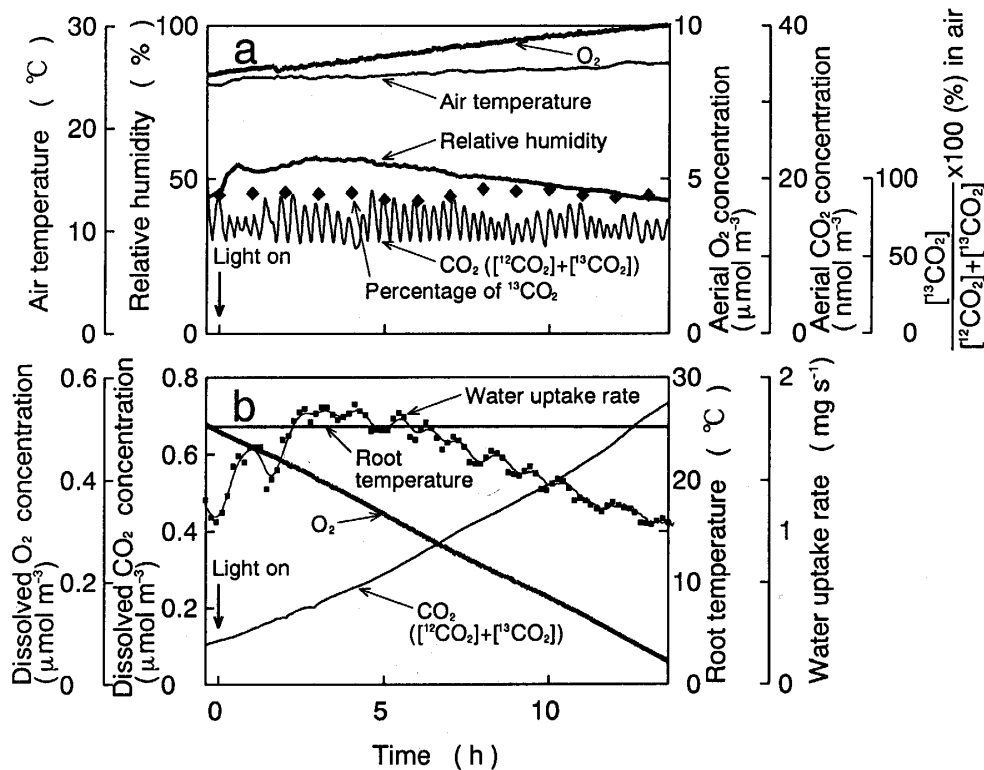


Fig. 2. Respective conditions of shoot environment (a) and root environment (b): Time course patterns of air temperature, relative humidity, aerial  $\text{O}_2$  and total  $\text{CO}_2$  ( $^{12}\text{CO}_2 + ^{13}\text{CO}_2$ ) concentrations, the percentage of  $^{13}\text{CO}_2$  in air, dissolved  $\text{O}_2$  and  $\text{CO}_2$  concentrations in nutrient solution, and water uptake rate in roots.

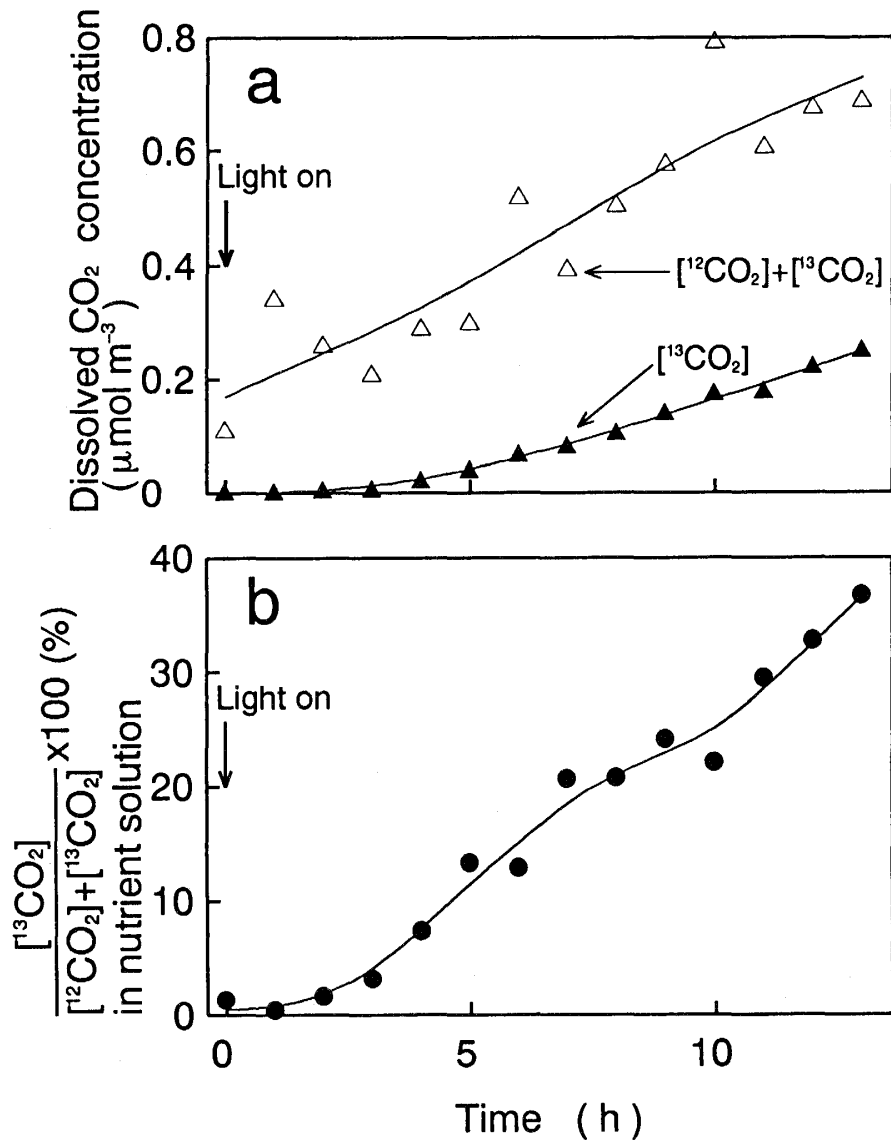


Fig. 3. Time course patterns of concentrations (a) of <sup>13</sup>CO<sub>2</sub> (<sup>13</sup>CO<sub>2</sub>), total CO<sub>2</sub> (<sup>12</sup>CO<sub>2</sub> + <sup>13</sup>CO<sub>2</sub>), and the percentage (b) of <sup>13</sup>CO<sub>2</sub> to total CO<sub>2</sub> in nutrient solution after lighting in the continuous <sup>13</sup>CO<sub>2</sub> supply to leaves: The values measured at an interval of 1 h are plotted with the fitted curves obtained by cubic spline functions.

gradually decreased with oscillation (Fig. 2b). These facts indicated physiologically active function in the plant used.

Figure 3 shows changes in concentrations (a) of <sup>13</sup>CO<sub>2</sub>, total CO<sub>2</sub> and the <sup>13</sup>CO<sub>2</sub> percentage (b) in the nutrient solution by light-on, in the case of the continuous <sup>13</sup>CO<sub>2</sub> supply to leaves. The total CO<sub>2</sub> increased in course of time, owing to the root respiration. At about 2 h after lighting under increased <sup>13</sup>CO<sub>2</sub>, the <sup>13</sup>CO<sub>2</sub> percentage in the solution started to increase and became 37% at 13 h.

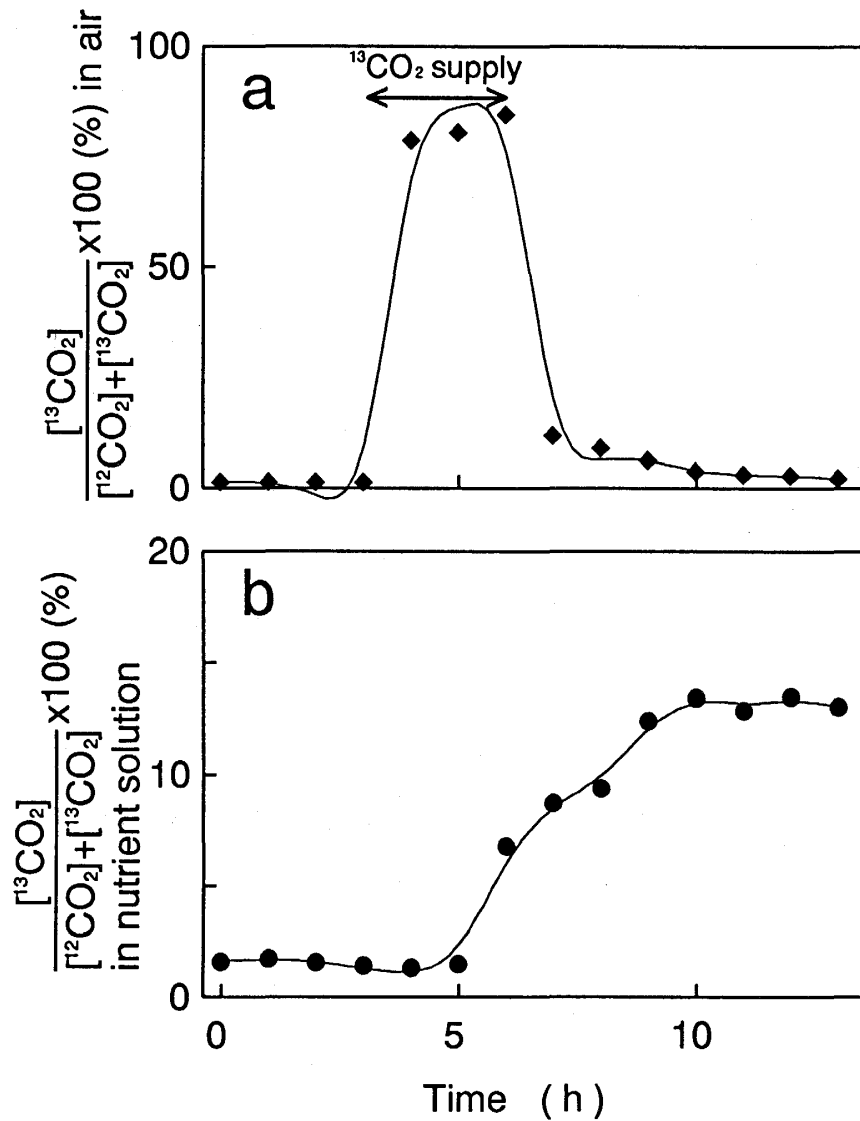


Fig. 4. Time course patterns of stepwise increase (a) in  $^{13}\text{CO}_2$  in air (shoot environment) and the step response (b) of  $^{13}\text{CO}_2$  release in roots to  $^{13}\text{CO}_2$  supply to shoot environment in continuous light, where percentages of  $^{13}\text{CO}_2$  to total  $\text{CO}_2$  ( $[^{12}\text{CO}_2]+[^{13}\text{CO}_2]$ ) in air and in nutrient solution are plotted at an interval of 1 h with the fitted curves obtained by cubic spline functions.

To analyze the dynamics of translocation of photosynthetic carbon to the roots,  $^{13}\text{CO}_2$  was stepwise supplied to leaves. Figure 4 shows the stepwise increase (a) in  $^{13}\text{CO}_2$  in air and the step response (b) of  $^{13}\text{CO}_2$  release in roots to the  $^{13}\text{CO}_2$  supply to leaves in continuous light. At the same time when  $^{13}\text{CO}_2$  was supplied to the shoot environment, the  $^{13}\text{CO}_2$  in air started to increase and became higher than 80% of total  $\text{CO}_2$  in air. When the  $^{13}\text{CO}_2$  supply to leaves was ceased, the  $^{13}\text{CO}_2$  in air decreased immediately to 12% within 1 h. On the

other hand, the initial value of the  $^{13}\text{CO}_2$  percentage in the nutrient solution was about 1% which was the same as the natural  $^{13}\text{C}$  isotopic abundance (1). At about 2 h after the  $^{13}\text{CO}_2$  supply to leaves, the  $^{13}\text{CO}_2$  appeared in the nutrient solution, which was translocated to the roots and respired. Thereafter the  $^{13}\text{CO}_2$  percentage in the solution increased to 13% and leveled off at about 7 h after the  $^{13}\text{CO}_2$  supply to leaves. McDougall (6) and Minchin and McNaughton (8) have reported that  $^{14}\text{CO}_2$  appears in root bathing solution within 20–60 min after photosynthesis in young wheat seedlings. Furthermore, Kouchi *et al.* have reported that  $^{13}\text{CO}_2$  can be found at 30–60 min after photosynthesis in soybean plant (4).

In this experiment, it was found that the delay of the time in translocation of newly fixed carbon in leaves to respiratory carbon release in roots is about 2 h in the cucumber plant at the 3 leaf stage.

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