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EVALUATION OF PHOTOASSIMILATE FLUX THROUGH A TOMATO PEDICEL

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ARAKI T., KITANO M. and EGUCHI H. *Evaluation of photoassimilate flux through a tomato pedicel*. BIOTRONICS 26, 21-29, 1997. An improved evaluation of photoassimilate flux through a pedicel of tomato plant (*Lycopersicon esculentum* Mill.) was examined in an application of ethylenediaminetetraacetic acid (EDTA) solution for collecting phloem exudate, where the cut end of the pedicel was immersed into the EDTA solution bath in a vial held on the pedicel. A part of the solution in the vial loaded with phloem exudate was sampled at a certain interval by using a micro syringe, and just after every sampling the solution bath was replenished with the fresh EDTA solution to keep the initial volume. Sugar concentration in the sampled solution and volumes of the solutions sampled and replenished were measured, and then photoassimilate flux exuded through the pedicel during the successive samplings was evaluated by analysis of sugar balance in the solution bath, where backflow through the pedicel was taken into account. The chelate effect of the EDTA solution was kept reliable for about a whole day, and photoassimilate flux was most enhanced in the solution bath of 20 mM EDTA and under the larger rates of replenishment with the fresh EDTA. Dynamic changes in the photoassimilate flux caused by lighting and leaf excision were evaluated clearly at a short sampling interval of 20 min. The results suggest that the improved EDTA method can be applied to short term analysis of dynamics of phloem translocation affected by change in pressure potential gradient through phloem from source leaves to the pedicel.

Key words: tomato fruit; *Lycopersicon esculentum* Mill.; photoassimilate flux; EDTA; phloem translocation; tomato pedicel.

INTRODUCTION

Dry matter accumulation into sink organs depends on phloem translocation of photoassimilate from source leaves. Phloem translocation has been studied by analyzing phloem exudate which is collected by using the aphid stylet method (6, 14), the incision method (3, 13, 15) or the EDTA (ethylenediaminetetraacetic acid) method (1, 2, 6, 8). In the former two methods, the small quantities of the sap are exuded and collected through a punctured or incised small part of phloem, but the exudation is ceased by callose formation induced in the wounded part of phloem. This callose formation, however, can be considered to

be prevented by treating the wounded part with a solution bath of a chelating agent such as EDTA which chelates with Ca^{2+} activating the callose formation, and the EDTA application to the petioles of excised leaves has been proved to enhance the phloem exudation by inhibiting the callose formation in sieve plate pores (1, 6).

In the previous paper (8), the EDTA method was applied to a tomato pedicel, where the cut end of the pedicel was immersed into the EDTA solution bath, and the flux of photoassimilate exuded through the pedicel cut end was evaluated from increment of photoassimilate in the solution bath. For more reliable evaluation of photoassimilate flux through a pedicel, it is desired to take account of sap backflow through the pedicel which can be caused under high evaporative demand and dynamics of plant water relations (4, 7, 9, 10).

The present paper deals with an improved evaluation of photoassimilate flux in the EDTA method applied to a tomato pedicel.

METHODS AND MATERIALS

Sampling of phloem exudate

A fruit was detached from the pedicel two hours prior to the start of evaluation of photoassimilate flux, and the cut end of the pedicel was pretreated with 20 mM EDTA solution adjusted to pH 7.0 with KOH: The cut end of the pedicel was immersed into the EDTA solution bath for two hours, and contents of cells exuded from the pedicel cut end was removed. After the pretreatment, a glass vial containing a certain volume (V_c) of the fresh EDTA solution was attached to the pedicel, where the pedicel cut end was immersed into the solution to a depth of about 1.5 cm. Figure 1 shows a schematic diagram and a

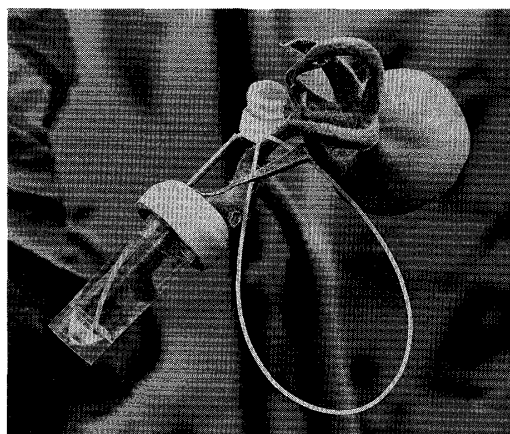
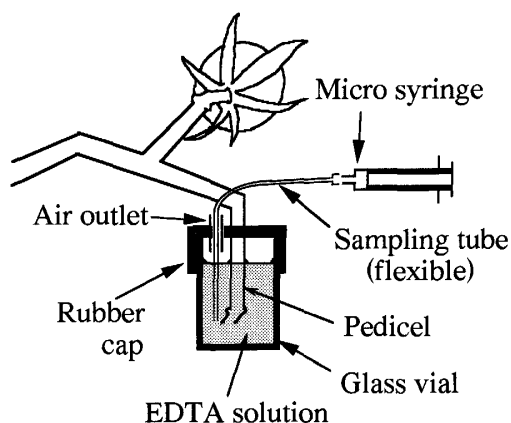


Fig. 1. Schematic diagram and photograph of a setup for collecting phloem sap exuded through a tomato pedicel into the EDTA (ethylenediaminetetraacetic acid) solution bath in a vial. A certain volume of the EDTA solution loaded with phloem exudate was sampled with a micro syringe through a flexible micro tube, and thereafter the solution bath was replenished with the fresh EDTA solution to keep the initial volume of the solution bath.

photograph of a setup for collecting phloem exudate. The glass vial was held on the pedicel by using a rubber cap with an air outlet, through which a flexible sampling micro tube was led to the solution bath. By using a micro syringe (500 μ l) attached to the sampling tube, a certain volume of the solution in the vial loaded with phloem exudate was sampled, and thereafter the fresh EDTA solution was supplied to the vial to keep the initial volume (V_C) of the solution bath.

Evaluation of photoassimilate flux

Figure 2 shows method for evaluation of photoassimilate flux (J_{SP}) during a sampling interval (Δt) between two successive sampling times of t_1 and t_2 . At every sampling time, the EDTA solution in the vial loaded with phloem exudate was sampled by a certain volume (ΔV_S), and then the vial was replenished with the fresh EDTA solution to keep the initial volume (V_C) of the solution bath. The replenished volume (ΔV_1 at t_1 and ΔV_2 at t_2) of the fresh EDTA solution was considered to be the sum of the sampled volume (ΔV_S) and the volume (ΔV_{P1} at t_1 and ΔV_{P2} at t_2) absorbed through xylem during Δt , that is $\Delta V_1 = \Delta V_S + \Delta V_{P1}$ at t_1 and $\Delta V_2 = \Delta V_S + \Delta V_{P2}$ at t_2 : The absorbed volume (ΔV_{P1} and ΔV_{P2}) seemed to be larger at lower xylem water potentials under higher evaporative demand from the environment. The initial volume (V_C) of the solution bath was set at 1000 μ l in this study, and the sampled volume (ΔV_S) and the replenished volumes (ΔV_1 and ΔV_2) were measured with the micro syringe at every sampling time (t_{1a} and t_{2a}). The sampled solution was filtered through a membrane with a pore size of 45 μ m, and soluble sugars (sucrose, glucose and fructose) were analyzed by using a high performance liquid chromatography (LC-10AD, SHIMADZU CORPORATION, Kyoto) with a stainless column packed with styrene-divinylbenzene copolymer (Shim-pack SCR-101N, SHIMADZU CORPORATION, Kyoto). The concentration (C_{1a} at t_1 and C_{2a} at t_2) of each sugar in the sampled solution was detected with a differential refractometer (RID-6A, SHIMADZU CORPORATION, Kyoto).

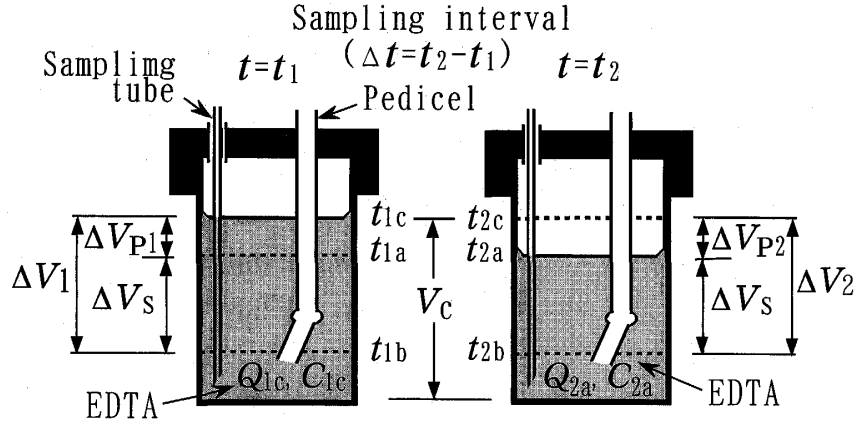
At the time (t_{1c}) when the vial was replenished with ΔV_1 of the fresh EDTA solution just after sampling of ΔV_S , sugar concentration (C_{1c}) in the vial was given by

$$C_{1c} = C_{1a}(V_C - \Delta V_1) / V_C \quad (1)$$

and then sugar content (Q_{1c}) in the vial was given by

$$Q_{1c} = C_{1c}V_C = C_{1a}(V_C - \Delta V_1) \quad (2)$$

During the succeeding sampling interval (Δt), the solution bath in the vial was loaded with sugar exuded through phloem, and further a small amount of the solution was absorbed through xylem under evaporative demand from the environment. At the next sampling time (t_{2a}) just before ΔV_S of the solution was sampled, sugar content (Q_{2a}) in the vial was given by



Sugar content

$$\begin{aligned} Q_{1c} &= C_{1c} \cdot V_c & Q_{2a} &= C_{2a} \cdot (V_c - \Delta V_{P2}) \\ &= C_{1a} \cdot (V_c - \Delta V_1) & &= C_{2a} \cdot (V_c - \Delta V_2 + \Delta V_S) \end{aligned}$$

Sugar balance during a sampling interval

$$Q_{1c} - \Delta Q_{P2} + J_{SP} \cdot \Delta t = Q_{2a}$$

Sugar loss absorbed through the pedicel

$$\Delta Q_{P2} = \Delta V_{P2} \cdot (C_{1c} + C_{2a}) / 2$$

Sugar flux exuded through the pedicel

$$J_{SP} = (Q_{2a} - Q_{1c} + \Delta Q_{P2}) / \Delta t$$

Fig. 2. Evaluation of photoassimilate flux exuded through a tomato pedicel by analysis of sugar balance in the EDTA solution bath in a vial, where the cut end of the pedicel was immersed into the solution bath. The solution loaded with phloem exudate was sampled by ΔV_S with intervals of Δt , and just after every sampling the solution bath was replenished with the fresh EDTA solution to keep the initial volume (V_c) of the solution bath: t_1 and t_2 , two successive sampling times; Δt , sampling interval ($t_2 - t_1$); t_{1a} , t_{1b} and t_{1c} , respective times just before sampling, after sampling and after replenishment at the sampling time of t_1 ; t_{2a} , t_{2b} and t_{2c} , respective times just before sampling, after sampling and after the replenishment at the sampling time of t_2 ; Q_{1c} , sugar content in the solution bath just after replenishment with the fresh EDTA solution at t_{1c} ; Q_{2a} , sugar content in the solution bath just before sampling at t_{2a} ; C_{1a} , sugar concentration at t_{1a} ; C_{1c} , sugar concentration at t_{1c} ; C_{2a} , sugar concentration at t_{2a} ; V_c , initial volume of the solution bath (the volume just after replenishment with the fresh EDTA solution at t_{1c} and t_{2c}); ΔV_S , sampled volume of the solution; ΔV_1 and ΔV_2 , volumes of the fresh EDTA solution replenished to the solution bath at t_{1c} and t_{2c} , respectively; ΔV_{P1} and ΔV_{P2} , volumes of the solution absorbed through xylem from the solution bath during Δt before the respective sampling times of t_{1a} and t_{2a} ; ΔQ_{P2} , sugar content in ΔV_{P2} absorbed through xylem during Δt from t_{1c} to t_{2a} ; J_{SP} , sugar flux exuded through the pedicel during Δt from t_{1c} to t_{2a} .

$$Q_{2a} = C_{2a}(V_C - \Delta V_{P2}) = C_{2a}(V_C - \Delta V_2 + \Delta V_S) \quad (3)$$

where the volume (ΔV_{P2}) absorbed through xylem was given by $\Delta V_2 - \Delta V_S$.

The sugar balance in the vial during the sampling interval between t_{1c} and t_{2a} was expressed as

$$Q_{1c} + J_{SP} \Delta t - \Delta Q_{P2} = Q_{2a} \quad (4)$$

where J_{SP} is the sugar flux exuded through phloem into the solution bath and ΔQ_{P2} is the sugar loss absorbed from the solution bath through xylem. ΔQ_{P2} was given by

$$\begin{aligned} \Delta Q_{P2} &= \Delta V_{P2}(C_{1c} + C_{2a}) / 2 \\ &= (\Delta V_2 - \Delta V_S) \{C_{1a}(V_C - \Delta V_1) / V_C + C_{2a}\} / 2 \end{aligned} \quad (5)$$

where the sugar concentration of the absorbed solution was approximately given as the mean concentration between t_{1c} and t_{2a} .

Based on the sugar balance of Eq. (4), the sugar flux (J_{SP}) exuded through phloem was expressed as

$$J_{SP} = (Q_{2a} - Q_{1c} + \Delta Q_{P2}) / \Delta t \quad (6)$$

By substituting Eqs. (2), (3) and (5) into Eq. (6), J_{SP} was evaluated at every sampling time by using measured values of C_{1a} , C_{2a} , V_C , ΔV_S , ΔV_1 and ΔV_2 . J_{SP} evaluated for each sugar, furthermore, was converted into the equivalent carbon flux (J_{CP}) by multiplying weight rate of carbon contained in a molecule of the sugar.

Plant materials

Tomato plants (*Lycopersicon esculentum* Mill. cv. Hausu-Momotaro) was potted in 8 l pots filled with vermiculite and were grown in a phytotron glass room at day/night temperature of 23/18°C and relative humidity of 70%, where the vermiculite in the pots was kept moistened enough by dripping complete nutrient solution. The plants were pinched at two leaves above the first truss before anthesis of the second truss. A few weeks after anthesis of the second proximal fruit on the first truss, the plants were used for evaluation of photoassimilate flux through the pedicel of the immature fruit at the stage of rapid sugar accumulation.

RESULTS AND DISCUSSION

Effect of EDTA concentration

For reliable evaluation of photoassimilate flux, it was essential to examine the chelate effect of EDTA applied in the tomato pedicel. The pedicel cut end was pretreated with 20 mM EDTA solution for two hours, and thereafter J_{CP} in

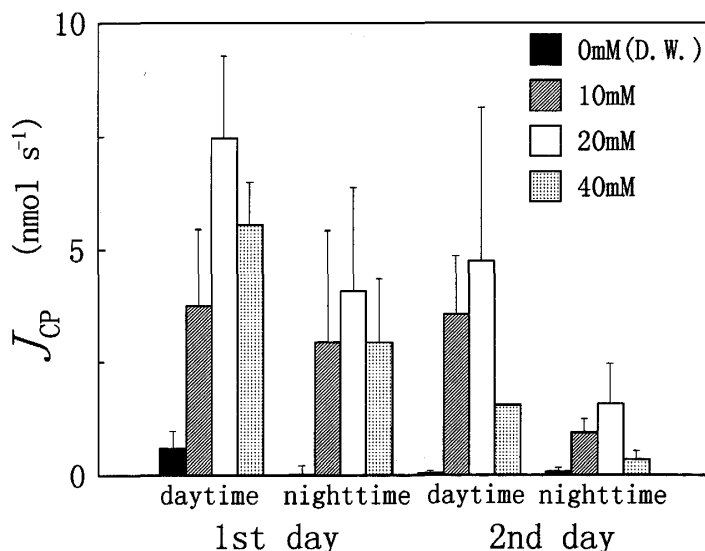


Fig. 3. Carbon fluxes through tomato pedicels of which cut ends were respectively immersed into $1000\mu\text{L}$ solution baths with different EDTA concentrations of 0 (distilled water), 10, 20 and 40 mM. The solutions in the respective baths loaded with phloem exudate were sampled by $250\mu\text{L}$ with intervals of 2h or 4h in a phytotron glass room. After every sampling, the solution baths were replenished with the fresh solutions of the respective EDTA concentrations to keep the bath volumes $1000\mu\text{L}$. Fluxes are expressed as mean values $\pm$ SD of six samples during the daytime (10:00–16:00) and the nighttime (22:00–06:00).

diurnal time courses was evaluated in the phytotron glass room by applying the solution baths with different concentrations of EDTA. Figure 3 shows carbon flux (J_{CP}) in the daytime (10:00–16:00) and the nighttime (20:00–06:00) during successive two days, where J_{CP} was evaluated by applying the solution baths with different EDTA concentrations of 0 (i.e. distilled water), 10, 20 and 40 mM under a sampling condition of V_C of $1000\mu\text{L}$, ΔV_S of $250\mu\text{L}$ and Δt of 2h or 4h. In the case of distilled water (0 mM EDTA), phloem exudation through the pedicel was inhibited in spite of the pretreatment with 20 mM EDTA, and J_{CP} remained remarkably low level. On the other hand, in the EDTA solution baths J_{CP} was clearly enhanced, and in particular the most enhanced J_{CP} was found in the 20 mM EDTA solution bath. In diurnal time course, J_{CP} was higher in the daytime than in the nighttime, but J_{CP} in each time was depressed in the second day. In the petiole of *Perilla crispera*, the effectiveness of the short time pretreatment with EDTA solution has been reported to be maintained even in the succeeding treatment with distilled water (6), but the tomato pedicel in this experiment required the continuous treatment with EDTA solution at the appropriate concentration. Thus, the continuous treatment with 20 mM EDTA solution was the most effective to enhance phloem exudation through the tomato pedicel, and its effectiveness was estimated to be maintained for about a whole day.

Furthermore, the chelate effect of EDTA to prevent callose formation in

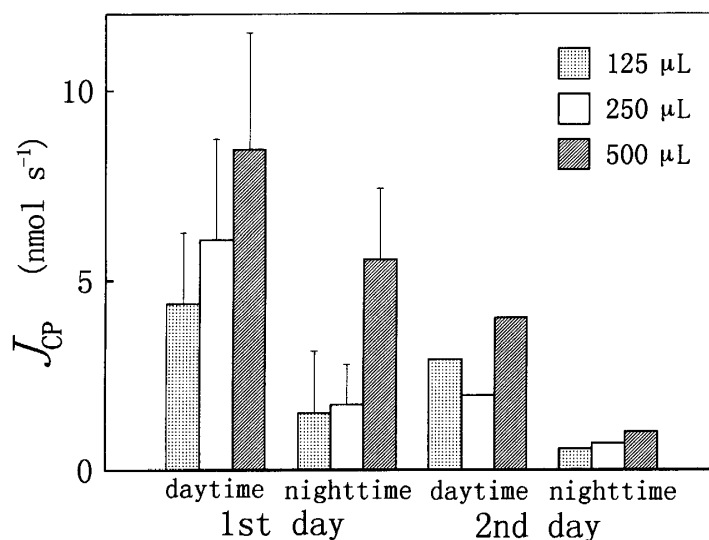


Fig. 4. Carbon fluxes through tomato pedicels of which cut ends were respectively immersed into 1000 μL EDTA solution baths with a concentration of 20 mM. The solutions in the respective baths loaded with phloem exudate were sampled by different volumes of 125, 250 and 500 μL with intervals of 2h or 4h in a phytotron glass room. After every sampling, the solution baths were replenished with the fresh 20 mM EDTA solution to keep the bath volumes 1000 μL . Fluxes are expressed as mean values $\pm$ SD of six samples during the daytime (10:00–16:00) and the nighttime (22:00–06:00).

sieve tubes was supposed to depend on the replenishment of the chelatable fresh EDTA to the solution bath. The volume (ΔV_1 and ΔV_2) of the fresh EDTA solution to be replenished at every sampling time was almost determined according to the sampled volume (ΔV_s) because the volume (ΔV_{P1} and ΔV_{P2}) absorbed through xylem was very small. Therefore, for examining the effect of replenishment of the fresh EDTA, J_{CP} was evaluated with different volumes of sampling under the condition of the 20 mM EDTA bath with V_C of 1000 μl and Δt of 2h or 4h. Figure 4 shows J_{CP} evaluated with each ΔV_s of 125, 250 and 500 μl in the phytotron glass room. J_{CP} was most enhanced under ΔV_s of 500 μl , where about a half part of the EDTA solution bath loaded with phloem exudate was exchanged for the fresh one. Thus, the more enhanced phloem exudation was found under the higher rates of the replenishment with the fresh EDTA solution at the optimum concentration of 20 mM.

Dynamics of photoassimilate flux

The performance of this EDTA method was examined on dynamic characteristic responsive to changing photoassimilate flux. The sampling interval (Δt) can be considered to be the most important determinant factor for dynamic characteristic of the method, that is, the shorter sampling intervals can bring the more sensitive responses to the changing flux. Therefore, dynamics of J_{CP} as affected by rapid changes in the conditions of light and area of source

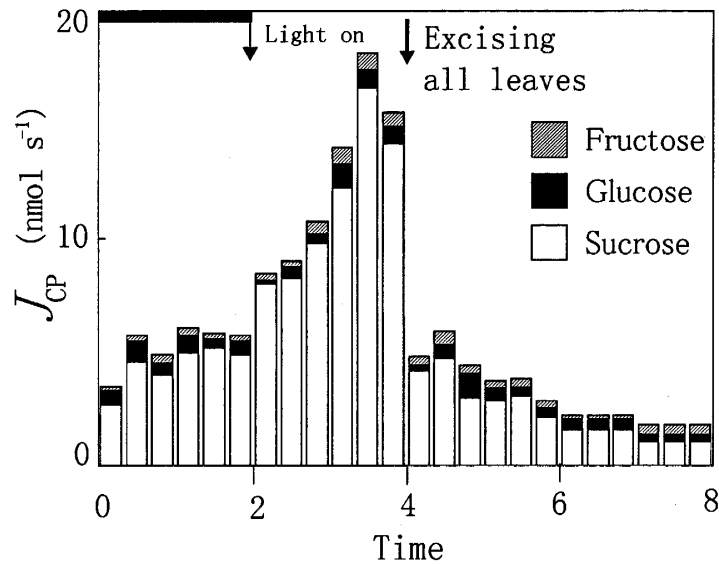


Fig. 5. Time course change in carbon flux through a tomato pedicel affected by lighting and leaf excision. The cut end of the pedicel was immersed into a 20 mM EDTA solution bath of 1000 μ l in an artificial light growth chamber. The solution loaded with phloem exudate was sampled by 500 μ l with intervals of 20 min, and just after every sampling the solution bath was replenished with the fresh EDTA solution to keep the bath volume 1000 μ l.

leaves was evaluated at a short sampling interval of 20 min by applying 20 mM EDTA solution with V_C of 1000 μ l and ΔV_S of 500 μ l: The plant was irradiated by metal halide lamps (DR400, TOSHIBA CORPORATION, Tokyo) at $PPFD$ of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under air temperature of 20°C and relative humidity of 70% in a growth chamber, and 120 min after lighting all leaves of the plant were excised. Figure 5 shows dynamics of J_{CP} affected by the lighting and the leaf excision. The principal sugar translocated through the tomato pedicel was sucrose, and dynamics of J_{CP} was dependent on sucrose flux. J_{CP} gradually increased by lighting and reached the maximum about 100 min after the start of lighting. Then the leaf excision caused abrupt drop in J_{CP} , and thereafter J_{CP} under no source leaves gradually decreased to the levels lower than those under the dark period before the leaf excision. Thus, the shorter sampling intervals made it possible to evaluate dynamics of photoassimilate flux caused by changes in the conditions (i.e. light and area) of source leaves. From Münch's pressure flow theory for phloem translocation (11, 12), these changes in source leaves can be estimated to affect phloem pressure flow from source leaves to sink organs, and it is conceivable that dynamics of J_{CP} evaluated by this EDTA method can reflect change in pressure potential gradient through phloems in the translocation path from source leaves to the pedicel. These results suggest that the improved method can be applied to analysis of short term dynamics of phloem translocation through a tomato pedicel.

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