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Araki, Takuya
Biotron Institute Kyushu University

Kitano, Masaharu
Biotron Institute Kyushu University

Eguchi, Hiromi
Biotron Institute Kyushu University

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T. ARAKI, M. KITANO and H. EGUCHI

Biotron Institute, Kyushu University 12, Fukuoka 812-8581, Japan

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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 $\text{Ca}^{2+}$ 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

![Schematic diagram and photograph of a setup for collecting phloem sap exuded through a tomato pedicel into the EDTA 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.](image)
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 (500μl) 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 \( \Delta t \) 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 \( \Delta V_S \), 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 \( \Delta V_1 \) at \( t_1 \) and \( \Delta V_2 \) at \( t_2 \) of the fresh EDTA solution was considered to be the sum of the sampled volume \( \Delta V_S \) and the volume \( \Delta V_{P1} \) at \( t_1 \) and \( \Delta V_{P2} \) at \( t_2 \): The absorbed volume \( \Delta V_{P1} \) and \( \Delta V_{P2} \) 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 μl in this study, and the sampled volume \( \Delta V_S \) and the replenished volumes \( \Delta V_1 \) and \( \Delta V_2 \) 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 45μm, 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 \( \Delta V_1 \) of the fresh EDTA solution just after sampling of \( \Delta V_S \), sugar concentration \( C_{1c} \) in the vial was given by

\[
C_{1c} = C_{1a} \frac{V_C - \Delta V_1}{V_C} \tag{1}
\]

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

\[
Q_{1c} = C_{1c} V_C = C_{1a} (V_C - \Delta V_1) \tag{2}
\]

During the succeeding sampling interval \( \Delta t \), the solution bath in the vial was loaded with sugar exuded through phloem, and 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 \( \Delta V_S \) of the solution was sampled, sugar content \( Q_{2a} \) in the vial was given by
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 $\Delta V_s$ with intervals of $\Delta t$, 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; $\Delta t$, 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 $t_{2c}$); $\Delta V_s$, sampled volume of the solution; $\Delta V_1$ and $\Delta V_2$, volumes of the fresh EDTA solution replenished to the solution bath at $t_{1c}$ and $t_{2a}$, respectively; $\Delta V_{P1}$ and $\Delta V_{P2}$, volumes of the solution absorbed through xylem from the solution bath during $\Delta t$ before the respective sampling times of $t_{1a}$ and $t_{2a}$; $\Delta Q_{P2}$, sugar content in $\Delta V_P$ absorbed through xylem during $\Delta t$ from $t_{1c}$ to $t_{2a}$; $J_{SP}$, sugar flux exuded through the pedicel during $\Delta t$ from $t_{1c}$ to $t_{2a}$.
where the volume ($\Delta V_{P2}$) 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

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

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

$$\Delta Q_{P2} = \frac{\Delta V_{P2} (C_{1c} + C_{2a})}{2} \frac{\Delta V_2 - \Delta V_S}{V_1 (C_{1a} (V_2 - V_S) / V_C + C_{2a})}$$

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

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

By substituting Eqs. (2), (3) and (5) into Eq. (6), $J_{SP}$ was evaluated at every sampling time by using measured values of $C_{1a}$, $C_{2a}$, $V_C$, $\Delta V_S$, $\Delta V_1$ and $\Delta V_2$. $J_{SP}$ 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 μL 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 μL 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 μL. Fluxes are expressed as mean values ± 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 μL, $ΔV_S$ of 250 μL 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 crispa, 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 2 h or 4 h 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±SD of six samples during the daytime (10:00–16:00) and the nighttime (22:00–06:00).

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

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leaves was evaluated at a short sampling interval of 20 min by applying 20 mM EDTA solution with $V_C$ of 1000 $\mu$l and $\Delta V_s$ of 500 $\mu$l. The plant was irradiated by metal halide lamps (DR400, TOSHIBA CORPORATION, Tokyo) at PPFD of 300 $\mu$mol m$^{-2}$ 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.
REFERENCES


