Dynamics of Photosynthate Loading in Strawberries
Affected by Light Condition on Source Leaves

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(Received October 4, 2016; Accepted November 16, 2016)

In greenhouse production of strawberries (Fragaria × ananassa × Duch.) during winter season in Japan, unsuitable climate condition (low solar radiation, low temperature, etc.) for strawberry growth causes serious problems such as yield depression and low quality of fruits. For sustainable production with high profitability, it is keenly desired to establish a system for efficient environmental control based on physiological functions of crops. Translocation of photosynthate from leaves to fruits is a major physiological determinant for size and sugar content of strawberries. It is therefore essential to clarify the response of photosynthate loading to surrounding environment. In this study, we focused on light condition which strongly influences the photosynthesis, and analyzed effects of irradiation on dynamics of photosynthate loading. Furthermore, aiming at the estimation of dynamics of photosynthate loading in cultivation field, we simulated daily amount of photosynthate loading by kinetic model using a saturable Michaelis-Menten component in combination with an unsaturable component obeying first-order kinetics.

Keywords : kinetic model, photosynthate partitioning, strawberry, sugar accumulation, sugar translocation

INTRODUCTION

Among the ecophysiological status that plants have, the translocation of photosynthate can be raised as one of the functions which has a great influence on the biomass and quality of the crops by accumulating photosynthate from leaves (source) to the subject organs for the harvest (sink) and directly control the auxetic growth in the sink organs along with the accumulation (David et al., 2014). Thus, for realizing the high-quality and high-yield for the crops, management of the cultivation environment based on the translocation dynamics and maintenance of the matter accumulation to the sink organs and auxetic growth in proper status are important (Kitano et al., 1998; Kitano and Araki, 2001; Araki et al., 2001).

The translocation mechanisms are composed of a variety of physiological functions and have complicated mechanisms. During the translocation, photosynthate generated on the source leaves become the sucrose which is the translocated sugar, and are loaded into the sieve tube by the sucrose transporter and the plasmodesmata (loading) (Sylvie et al., 1999). Then, the sucrose flown into the sieve tube is transported by the concentration gradient and the flow generated due to the water permeation and inflow from the xylem within the sieve tube which links between the source and the sink (Hölttä et al., 2006; Tsyn-kay et al., 2014), and is loaded to the sink organs by the active transport via the membrane transport protein (unloading) (Lalonde et al., 2003). Although the physical characteristics play a important role in terms of the transport in the sieve tube (Jonas et al., 2011), the loading from the source leaves to the sieve tube and the unloading from the sieve tube to the sink fruit are largely influenced by the environmental factors such as light environment, temperature, humidity, CO₂ concentration and wind velocity (Remi et al., 2013). Due to the complexity of these translocation mechanisms, it is difficult to measure the quantitative information in the translocation dynamics, and therefore, the number of studies conducted to evaluate the translocation process against environmental response is extremely low. Quantitative evaluation in the response of the translocation dynamics against the environmental actions are required in order to study the management of the cultivation environment based on the translocation dynamics.

In this study, we focused on the loading among such a complicated translocation mechanisms. The loading of the sucrose generated as the translocated sugar on the source leaves is carried out by the passive transport in line with the concentration gradient between the mesophyll cells and phloem cells through the plasmodesmata and by the active transport due to the sucrose transporter (Aart et al., 1992). The major limiting factor for the loading is the concentration of the sucrose in the source leaves, therefore it is inferred that it depends on the amount of sucrose generated from the photosynthesis during the day. Under this circumstances, we focused on the light environment as a factor influencing on the photosynthesis and studied the im-
pact which the photosynthesis carried out in the source leaves has on the sucrose concentration and the amount of the loading in the source leaves by providing processing treatments per each light condition. Furthermore, aim to achieve the quantitative evaluation of the loading dynamics at the production site where is difficult to learn the loading dynamics since the destruction of plants or the measurement with expensive measurement equipment are difficult, we tried to create the model to estimate the loading dynamics from the light condition which can be measured easily.

MATERIALS AND METHODS

Plant materials
Strawberry plants (Fragaria × ananassa Duch. ‘Benihoppe’) were grown by pot cultivation in phytotron (temperature of 20 ± 1°C, humidity of 70 ± 5%) located at Kyushu University Biotron Application Center. At the beginning of June 2013, seedlings selected from mother stocks were planted in plastic pots (0.15 m diameter; 2.6 L volume) filled with mixed substrate (peat moss: coconut shells: charcoal 3:5:2 [v/v/v]) and were grown on a seeding bench. OK-F-1 nutrient solution (Otsuka Chemical, Japan) with an electrical conductivity of 0.6 dS m⁻¹ was supplied at a rate of 300 mL plant⁻¹ day⁻¹ divided into 5 times at 9:00, 11:00, 13:00, 15:00, and 17:00. In the summer season, nutrient supplementation was halted to induce anthesis, with only water supplied. Seedlings were transplanted to substrate-filled cultivation pots on September 1, 2013, and were then grown under nutrient solution supplementation. Substrates and nutrient solutions were the same as those used for seedling cultivation, with approximately 3 L of substrate used per plant.

Experiment treatments
As experiment treatment, shaded treatment, LED supplemented treatment and control treatment were provided (Fig. 1). We have cultivated 6 strawberry plants for each processing treatment with 90% shading on the shaded treatment by using the cheesecloth and under natural light on the control treatment. For the LED supplemented treatment, we have provided LED lamp unit (LLM0312A) coupled to an external power supply (LLP0019A, Stanley Electric Co., Ltd., Japan) at the height of 30 cm from the plant bases for ensuring sufficient amount of lights for growing strawberries (Hidaka et al., 2013). The LED units have been applied for 12 h from 6:00 to 18:00.

Measurement of environmental conditions
During the experiments, we measured the temperature and CO₂ concentration of ambient air around the canopy on each treatment. T-thermocouples were used to measure air temperature and CO₂ sensor (GMT220, Vaisala Co., Ltd., Finland) were used to measure CO₂ concentration. Furthermore, we also measured photosynthetic photon flux density (PPFD) for the each treatment using a quantum sensor (PAR-02, PREDE Co., Ltd., Japan). The data were recorded using a data logger (GL820, Graphite Corporation, Japan) at 10-min intervals.

Measurement of leaf photosynthesis
We measured diurnal changes of leaf photosynthetic rates in each treatment at every hour from 8:00 to 18:00. The measurements were carried out by a portable photosynthesis and fluorescence system (LI-6400XT, LI-COR Inc., USA) using chamber head with natural light window under the ambient air temperature, CO₂ concentration and relative humidity conditions. Upon the measurement, 3 plants were selected from each treatment and the rates were measured for the third expanded leaves.

Measurement of sucrose concentration
We have measured the sugar concentration in leaves for the sucrose which is the translocated sugar as the photosynthate. As the source leaves of the strawberry, we have collected a sample from the each third expanded leaf of the 6 crops in each treatment by using 6 mm diameter punch with avoiding the major vein. In addition, we have performed the quantitative analysis on the collected samples using High-Performance Liquid Chromatography (HPLC, SHIMADZU CORPORATION, Japan) (Rybak-Chmielewska, 2007; Dimins et al., 2008; Beitane et al., 2013; Liga et al., 2014). The quantitative analysis found the sucrose concentration per 1 mL of extracted solution. The molar concentration of the sucrose per unit area of the source leaf has been calculated from the acquired
concentration values divided by the sucrose molar mass (342.30 g mol$^{-1}$) and the area of the collected samples.

**Sucrose loading model**

In this study we focused on the loading of the translocated sugar sucrose. The loading is performed by the active transport through the sucrose transporter and the passive transport through the plasmodesmata. The active transport can be understood as the enzymatic reaction with considering the sucrose which is the transport solute as a substrate and the sucrose transporter which is the transport protein as the enzyme. The rate equation of the active transport can be described in the Michaelis-Menten equation which is used as the rate equation for the enzymatic reaction depending on the substrate concentration. The passive transport is performed based on the concentration gradient of the sucrose through the structure called the plasmodesmata which is a passageway between two adjacent cells. The rate equation of the passive transport can be described with the first order reaction rate equation which is proportionate to the concentration gradient of the sucrose between the phloem and the mesophyll cells. The formula of the loading model is shown in the equation (1) which is a sum of the active transport terms and the passive transport terms (Tom et al., 2012).

$$ Load = \frac{V_{\text{max}}}{K_{M,L} + Su} + k_1 Su $$

where $Load$ is loading rate (mmol m$^{-2}$ h$^{-1}$), $V_{\text{max}}$ and $K_{M,L}$ are the Michaelis-Menten constants (mmol m$^{-2}$ h$^{-1}$ and mmol m$^{-2}$, respectively), $Su$ is sucrose concentration in leaf (mmol m$^{-2}$) and $k_1$ is the first-order rate constant (h$^{-1}$).

**RESULTS AND DISCUSSION**

Figure 2 shows diurnal changes of temperature and CO$_2$ concentration (b) measured at each treatment. During day time, in LED treatment, air temperature was higher by up to 1°C than in other treatments. During nighttime, compared with control treatment, shaded treatment was higher by about 0.5°C and LED treatment was lower by 0.5°C, respectively. No difference in CO$_2$ concentration was observed between the treatments.

Figure 3 shows diurnal changes of PPFD and photosynthetic rate of strawberry leaves in each treatment. In shade treatment, PPFD made transition at around 10% of that in control treatment, whereas PPFD in LED treatment made transition continuously at higher constant level during light supplement. Photosynthesis rate in shade treatment shifted at around 0 during day time, while significant effect of light supplement was observed in LED treatment for 4 h after sunrise and before sunset, respectively. Integrated PPFD for 12 h during day time and integrated amount of photosynthesis is shown in Fig. 4. Integrated amount of photosynthesis was calculated by the integration of Fig. 3b in each treatment. As few photosynthesis was performed in shade treatment due to less light, integrated amount of photosynthesis for 12 h is almost 0. In control treatment where cultivation was performed under natural sunlight, variation was observed in PPFD integrated value from day to day as observed similarly in integrated amount of photosynthesis per day. In LED treatment, with an effect of LED supplement light, PPFD integrated value and integrated amount of photosynthesis per day both were at almost constant level during the experiment period. In comparison with control treatment, about 2.5 to 3 times of PPFD of light was applied and 1.7 to 2 times of photosynthesis was performed in LED treatment.

Figure 5 shows transition of sucrose concentration per unit area for every 12 h in a source leaf of strawberry. Leaf sucrose concentration in shade treatment made a transition constantly at low value level. On the other hand, leaf sucrose in LED treatment significantly increased during day
time and greatly decreased during night showing almost the same leaf sucrose concentration in the morning compared with that in control treatment.

Figure 6 shows amount of change in leaf sucrose concentration during the daytime from 6:00 to 18:00 and during the nighttime from 18:00 to 6:00 the following morning. Positive value of amount of change during the daytime indicates sucrose accumulation in the leaves, and negative value of amount of change during the nighttime indicates sucrose loading from the leaves. The greater is the integrated amount of photosynthesis per day, so is the amount of sucrose accumulation in the leaf, and the greater is the amount of sucrose accumulation, so is the loading amount. While no difference in integrated amount of photosynthesis per day is observed between experiment days, some variations are observed in changed amount of leaf sucrose concentration. It was caused by the impacts of environmental factors other than light and interaction with leaf sugar other than sucrose. The reason why amount of sucrose loading during day time in shade treatment surpassed amount of sucrose accumulation is considered that the amount of sucrose accumulation decreased due to extremely small amount of light during day time.

Figure 7 shows a relationship between amount of sucrose accumulation and amount of sucrose loading in a source leaves. The following equation was obtained as a result of curve regression by least-square method.

\[
\text{Load} = \frac{56.3}{52.5 + Su} + 0.261Su \\
R^2 = 0.739
\]

From a loading model obtained by fitting, loading amount during night was estimated based on accumulation amount of sucrose as a translocation sugar during day time (Fig. 8a). In addition, a relationship of actual measured loading value with estimated value based on a model is shown in Fig. 8b. Significantly high correlation was observed as indicated by \( R = 0.953 \). Transition of leaf sucrose concentration for every 12 h was estimated using estimated sucrose loading amount (Fig. 9). In estimating transition for every 12 h, actual measured values were used for amount of sucrose accumulation. While some discrepancies were observed, dynamics of leaf sucrose concentration resulted in almost the same as actual measured values.

We tried to estimate amount of sucrose accumulation in a source leaf from integrated PPFD per day (Fig. 10) aiming at estimating loading amount from light environment. Since amount of sucrose accumulation is determined by photosynthetic reaction in a source leaf, approximation was performed using Michaelis-Menten equation as a regression curve. As a result of curve fitting, a high correlation was recognized as indicated by \( R = 0.877 \). Amount of sucrose accumulation during day time was estimated from integrated PPFD per day using obtained regression equation (Fig. 11). With almost constant integrated PPFD per

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**Fig. 4** Daily integrated PPFD (a) and daily integrated photosynthetic rate (b) in LED treatment, control treatment and shade treatment during clear days (Sep. 7–10, 2013).

**Fig. 5** Diurnal changes in sucrose concentration of fully expanded third leaflet under each treatment during clear days (Sep. 7–10, 2013).

**Fig. 6** Daily changes of sucrose concentration of fully expanded third leaflet under each treatment during clear days (Sep. 7–10, 2013). Positive value means the sucrose accumulation in strawberry leaves and negative value means the sucrose loading from leaves.

**Fig. 7** Relationship between amount of sucrose accumulation in strawberry leaves and amount of sucrose loading from leaves.
day observed in each experiment day in each treatment, amount of sucrose accumulation was recognized as a constant value similarly. In case of estimation based on integrated PPFD per day, a difference is generated between actual measured value and estimated value due to presence of variation in photosynthesis rate depending on the time zone in addition to error cause generated by estimation based on integrated amount of photosynthesis per day.

Accumulation and loading of leaf sucrose concentration for every 12 h were estimated using amount of sucrose accumulation estimated from integrated PPFD per day and loading amount obtained from a loading model (Fig. 12). In comparison of transition of sucrose concentration between actual measured value and estimated value, difference in concentration between control and light supplement segments at 18:00 became smaller and difference from
actual measured value in concentration at 6:00 after loading during night was seldom recognized in all treatments.

In this study, we focused on sucrose loading, and analyzed effects of light condition on loading. Furthermore, aiming at the estimation of dynamics of sucrose loading in cultivation field, we estimated amount of sucrose loading aiming at the estimation of dynamics of sucrose loading in cultivation field, we estimated amount of sucrose loading during night was seldom recognized in all treatments.

![Fig. 12](image)

**Fig. 12** Diurnal changes in measured sucrose concentration (a) and estimated sucrose concentration (b), which was estimated from PPFD, of strawberry leaves in LED treatment, control treatment and shade treatment during clear days (Sep. 7–10, 2013).

ACKNOWLEDGMENTS

The present study was supported by the Grant-in-Aid for Scientific Research (No. 23380150 and No. 15J07916) from the Japan Society for the Promotion of Science.

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