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SHORT COMMUNICATION

Starch serves as an overflow product in the regulation of carbon allocation in strawberry leaves in response to photosynthetic activity

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Abstract

The carbon allocation in source leaves between sucrose and starch is an important mechanism that affects plant productivity. We previously found that strawberry plants accumulate starch in response to excess carbon supply from photosynthesis compared with translocation and sucrose storage capacity in source leaves. However, because these data were acquired from three separate cultivation seasons in field conditions, seasonal impacts could not be ruled out. Therefore, herein, we aimed to investigate the role of starch in carbon allocation in strawberry leaves and to explore whether the relationship between sucrose and starch reported in our previous study is an inherent characteristic that is independent of seasonal variations. To prevent seasonal influences, carbohydrate dynamics in strawberry leaves were studied under controlled environmental conditions with high (High) and low (Low) photosynthetic activity. During the day, both sucrose and starch concentrations increased in the High treatment, but starch concentration increased only marginally in the Low treatment. Furthermore, starch production was enhanced in the High treatment when sucrose concentration exceeded 150 mmol C m^{-2}. Consistent with previous findings, the current findings indicated that photosynthetically fixed carbon is initially allocated to sucrose; however, when photosynthetic activity increases and leaf sucrose concentration exceeds its storage capacity, the excess carbon is then allocated to starch. This study provides strong evidence that, regardless of season, starch serves as an overflow product with sucrose storage capacity as a threshold during carbon allocation in strawberry leaves.

Keywords Overflow hypothesis · Carbohydrate metabolism · *Fragaria* × *ananassa* · Sucrose storage capacity · Translocation

Introduction

Carbon allocation, a process that significantly affects crop productivity, is the distribution of photosynthetically fixed carbon into multiple metabolic pathways and the synthesis of various forms of carbohydrates (Taiz et al. 2018). Sucrose and starch are the most common photosynthetic end products (Lunn and Hatch 1995; McClain and Sharkey 2019). In many species, including the model plant *Arabidopsis*, carbon from atmospheric CO_2 is fixed via the Calvin–Benson cycle in chloroplasts, whose intermediate products (triose phosphate) remain in the chloroplasts for starch synthesis or are exported to the cytosol for sucrose synthesis. Sucrose synthesized in the cytosol of "source" leaves is translocated to "sink" organs via phloem throughout the day and is used for growth, storage, and metabolism in the sink organs (Geiger and Servaites 1994; Smith and Stitt 2007; Stitt and Zeeman 2012). Meanwhile, starch is stored in source

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leaves during the day and subsequently degraded to sucrose and remobilized at nighttime for translocation, growth, and maintenance. Because starch accumulation competes with sucrose synthesis for translocation, the carbon allocation between sucrose and starch in leaves determines plant productivity (MacNeill et al. 2017). Although carbon allocation is an important process in sink organs, in this study, we focused on the carbon allocation in leaves as the fundamental process of plant growth.

Regarding the regulation of carbon allocation between sucrose and starch in leaves, two hypotheses have been proposed (Stitt et al. 2010; Mengin et al. 2017). The first hypothesis (overflow hypothesis) (Cseke et al. 1984; Stitt 1990) proposes that starch is synthesized as an overflow product when the photosynthetic rate exceeds the translocation rate and sucrose storage capacity in source leaves. The second hypothesis proposes that starch accumulates in leaves to maintain a consistent supply of sucrose and avoid carbon starvation at night. The second hypothesis has been emphasized in numerous studies on *Arabidopsis* (Smith and Stitt 2007; Gibon et al. 2009; Graf and Smith 2011; Sulpice et al. 2014). These studies demonstrated that even under conditions of low photosynthetic activity, *Arabidopsis* accumulated a portion of photosynthetically fixed carbon as starch, and the starch reserve was almost, but not entirely, depleted by circadian regulation at the next dawn. Although various biochemical reactions related to the regulation of sucrose and starch synthesis are known (Stitt et al. 2010; Taiz et al. 2018), the mechanisms by which carbon allocation between sucrose and starch is regulated in both hypotheses are not entirely clear.

Furthermore, the role of sucrose and starch in carbon allocation differs substantially across species (Streb and Zeeman 2012; Smith and Zeeman 2020). As previously mentioned, *Arabidopsis* predominantly accumulates starch in source leaves but rarely any sucrose (Annunziata et al. 2018). In contrast, cereal crops such as rice, wheat, and barley predominantly accumulate and use sucrose as a nighttime resource instead of starch (Gordon et al. 1980; Rösti et al. 2007). The ratio of starch to sucrose in source leaves reflects the differences in their roles and is closely related to the regulation of carbon allocation (Dong and Beckles 2019).

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the world's most important horticultural crops. The amount of carbohydrates, which are translocated from source leaves to fruits, influences yield and fruit quality. As a result, the carbon allocation in leaves, which determines the available carbon for translocation, is an important phase in strawberry production. We previously found that strawberry accumulated sucrose and starch throughout the day and used both at night and that the synthesis of starch was remarkable when the sucrose concentration per unit leaf area was greater than 150 mmol C m^{-2} (Nakai et al. 2022), and these results were consistent with the hypothesis that starch is an overflow product (Cseke et al. 1984; Stitt 1990). However, these results were obtained from three cultivation periods with different day lengths in the field. It has been proposed that the carbon allocation to starch in some species, like *Arabidopsis*, varies with day length (Stitt and Zeeman 2012; Mengin et al. 2017). Therefore, it is possible that the environmental response of the carbohydrate metabolism of sampled strawberry plants varies according to season; consequently, our previous study (Nakai et al. 2022) was insufficient to confirm how sucrose and starch dynamics are related to photosynthetic activity.

Herein, we aimed to determine whether the relationships between sucrose and starch dynamics in leaves suggested in the previous study (Nakai et al. 2022) were an inherent characteristic in strawberry plants and whether starch served as an overflow product in the regulation of carbon allocation. To avoid seasonal effects, experiments were conducted under controlled environmental conditions using strawberry plants grown within the same cultivation period. The concentrations of the most abundant carbohydrates in strawberry leaves— sucrose, glucose, fructose, and starch (Nishizawa 1994)— were measured in conditions of high or low photosynthetic activity, and the relationship between sucrose and starch dynamics was investigated.

Materials and methods

Plant materials and growth conditions

Strawberry plants (*Fragaria* × *ananassa* Duch. 'Fukuoka S6') were grown under natural light conditions in a greenhouse (20 m long × 8 m wide × 4 m high) located at the Ito Plant Experiment Field & Facilities, Faculty of Agriculture, Kyushu University, Japan. The initial temperature of greenhouse ventilation was set to 22 °C, and a heater was operated to maintain it above 8 °C. The nursery plants were transplanted into plastic pots (28.0 cm long × 14.5 cm wide × 11.8 cm high) filled with M-1 substrate (Kaneya Co., Ltd., Aichi, Japan) on September 24, 2021; 16 plants were planted (2 in each pot) and then placed on cultivation beds (1.2 m high). The plants were supplied with the nutrient solution (OK-F-1, OAT Agrio Co., Ltd., Tokyo, Japan; an electrical conductivity = 0.6 dS m^{-1}; N:P:K = 75:40:85 mg L^{-1}) at a rate of 200 mL d^{-1}/plant. Each plant had eight fully expanded leaves and a seven fruits or flowers during the experiments.

Experimental conditions and treatments

The experiments were conducted on December 15 and 23, 2021, in a plant growth chamber (HNM-S11, Koito Electric Industries, Ltd., Shizuoka, Japan; growth area: 60.0 cm long × 90.0 cm wide × 90.0 cm high) that can control air temperature and relative humidity. Nine dimmable LED floodlights (HMFD45EW1SV12H-RM (40Y), Kyoritsu Densho Co., Ltd., Miyazaki, Japan) were used as the light source (Online Resource Fig. S1).

The light condition was controlled to provide two treatments: one with high photosynthetic activity treatment (High) that replicated the greenhouse light condition on a clear day in December and another with low photosynthetic activity treatment (Low) that replicated a rainy day in December. The maximum photosynthetic photon flux density (PPFD) was set to approximately 970 and 300 µmol m^{-2} s^{-1} for the High and Low treatments, respectively, by manually operating the dimmers in a stepwise manner from dawn to dusk. Both treatments had the same dawn/dusk times of 7:15/17:15, air temperature of 20 °C during the day (7:15–17:15) and 12 °C at night (17:15–7:15 the following day), and relative humidity of 70% during the entire day.

Measurement of environmental conditions and leaf temperature

PPFD was measured using a light quantum meter (PAR-02D, Prede Co., Ltd., Tokyo, Japan) placed at a canopy height of 35.0 cm from the growth area floor of the chamber. Air relative humidity and ambient air CO_2 concentration were measured using a temperature-humidity sensor (HMP60, Vaisala, Finland) installed in a forced ventilation shelter (RSVH01A1203, SCE, Inc., Hokkaido, Japan) and a CO_2 sensor (GMP252, Vaisala, Finland), respectively, which were placed at a height of 50 cm (above the canopy). Leaf temperature was measured using a T-type thermocouple (0.1-mm diameter) fixed to an arbitrary leaf surface. Data were logged at 5-s intervals using a data-logger (CR1000X, Campbell Scientific, USA) and 5-min means were automatically stored in it.

Estimation of photosynthetic activity

To ensure that the treatments were appropriately set, the photosynthetic rate in a single leaf was used as an indicator of photosynthetic activity. We used our previously reported estimation model of single-leaf photosynthesis under sunlight (Nakai et al. 2022) with some modifications. The model was based on the biochemical photosynthesis model (Farquhar et al. 1980) and the stomatal model (Medlyn et al. 2011). Regarding the modification from our previous study, the actual measured leaf temperature was used instead of the estimated value using the energy balance equation of the leaf, as LED produces less heat than sunlight. The estimation was performed using the R package "plantecophys" (version 1.4.4, Duursma 2015) in R version 4.2.2 (R Core Team 2022). The photosynthetic model parameters used for estimation were obtained using a portable open gas-exchange system (LI-6400XT, LI-COR, USA) as described by Kimura et al. (2020) and Nakai et al. (2022) and are shown in Online Resource Table S1.

Leaf sample processing and analysis of carbohydrate concentrations

Each treatment experiment was conducted on eight plants that had been transferred to the plant growth chamber the night before the experiment. A total of 48 fully expanded leaves were selected from the 8 plants, and 1 leaf disk (6-mm diameter, 28.3 mm^2) of every selected leaf was sampled from the intercostal regions (i.e., the lamina between major veins) throughout the day. Sampling was performed 10 times within 24 h at 2-h intervals during the day (7:15, dawn; 9:15; 11:15; 13:15; 15:15) and at 2- or 4-h intervals during the night (17:15, dusk; 19:15; 23:15; 3:15; 7:15). To ensure that sufficient amounts of carbohydrates were available for analysis, the 48 leaf disks collected at each sampling time were grouped into sets of 12, i.e., 4 biological replicates were obtained in each treatment. All samples were stored at − 80 °C until further analysis.

The concentrations of soluble sugars (sucrose, glucose, and fructose) and starch were quantified using freeze-dried powder of the leaf disks. Soluble sugars were assayed as described by Nakai et al. (2022). Briefly, freeze-dried samples were ground, and then extracted three times using 80% (vol/vol) ethanol at 82 °C as described by Nishizawa (1994). The supernatants were combined, and the ethanol was evaporated under vacuum. Soluble extracts were resuspended in distilled water, filtered (0.45 µm), and then used for high-performance liquid chromatography analysis as described by Nakai et al. (2022). Starch concentration was determined enzymatically using the Total Starch Assay kit (K-TSTA, Megazyme, Ireland) following the manufacturer's protocol with minor modifications according to the sample size. Following ethanol extraction, the pellets were resuspended in 2 mL of sodium acetate (100 mM, pH 5.0), and the starch was then converted to glucose by adding of 25 µL each of α-amylase (2500 U mL^{-1}) and amyloglucosidase (3300 U mL^{-1}). Glucose concentrations were determined using a spectrophotometer (UV-1800, Shimadzu Corp., Japan) set to 510 nm and the Megazyme GOPOD reagent. Starch concentrations were calculated based on a glucose standard curve.

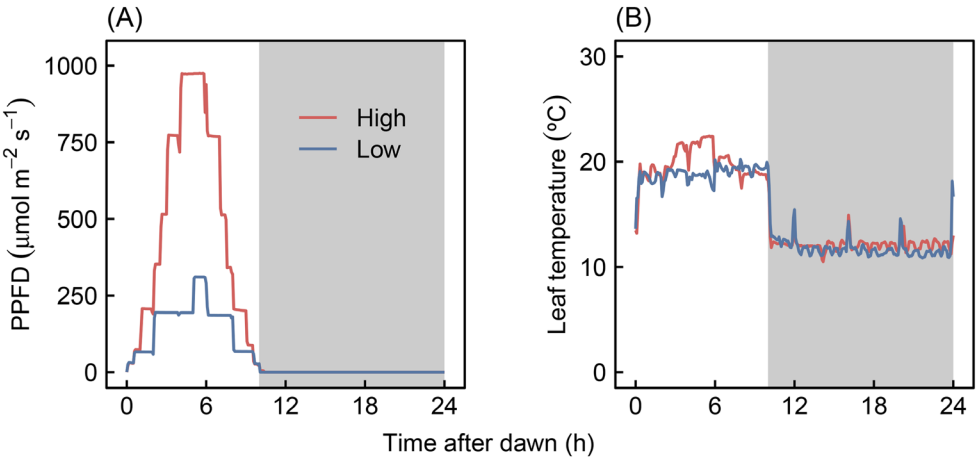

Fig. 1 Diurnal changes in photosynthetic photon flux density (PPFD) **(A)** and leaf temperature **(B)** from dawn in two treatments: high photosynthetic activity treatment (High; red line) and low photosynthetic activity treatment (Low; blue line). Shaded regions represent the nighttime

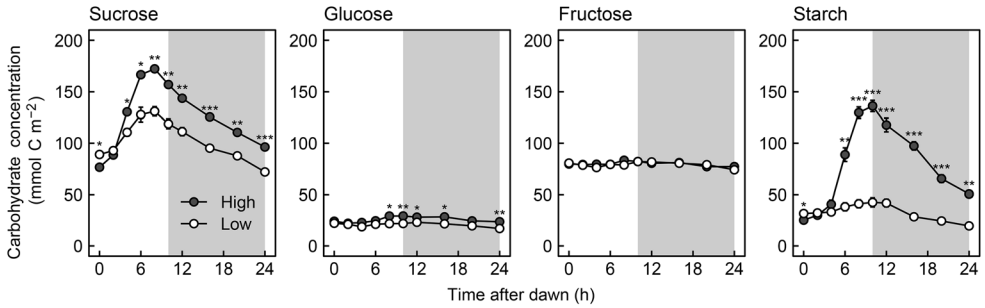

Fig. 2 Diurnal changes in leaf carbohydrate concentrations from dawn in two treatments: high photosynthetic activity treatment (High; closed circles) and low photosynthetic activity treatment (Low; opened circles). Shaded regions represent the nighttime. Error bars represent the standard error of the mean values ($n=4$). Significant differences between treatments are indicated by asterisks (Student's t-test): $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$

Statistical analyses

Statistical analyses were performed using R version 4.2.2 (R Core Team 2022). The carbohydrate concentration data were statistically expressed as means ± standard error ($n=4$) and subjected to Student's t-test to identify significant differences between two treatments at each sampling time point.

Results and discussion

Light conditions and leaf temperature

To assess how photosynthetic activity affects leaf carbohydrate dynamics, we conducted sampling experiments in two treatments with different light conditions. Figure 1 shows the diurnal changes in PPFD (A) and leaf temperature (B) in the two treatments. The daily maximum PPFD for the High and Low treatments was approximately 970 and 300 µmol m^{-2} s^{-1}, respectively; however the PPFD was generally higher in the High treatment than the Low treatment. The leaf temperature mainly varied depending on the air temperature set (day: 20 °C; night: 12 °C). In the High treatment, PPFD had a minimal effect on leaf temperature when its value exceeded 750 µmol m^{-2} s^{-1}. The local fluctuations in leaf temperature observed throughout the day, such as 4 and 12 h after dawn, were probably caused by the opening and closing of the chamber's door for sampling.

Single-leaf photosynthetic rate

The estimated photosynthetic rate changed mainly depending on PPFD (Oneline Resource Fig. S2). The photosynthetic rate remained generally higher in the High treatment than in the Low treatment throughout the day, indicating that the treatments were set properly.

Leaf carbohydrate concentrations

The diurnal changes in leaf carbohydrate concentrations in the two treatments reflected a difference in photosynthetic activity (Fig. 2). The sucrose concentration in the High treatment was approximately 75 mmol C m^{-2} at dawn. It immediately increased at dawn, peaked at 170 mmol C m^{-2} 4 h before dusk, and then decreased in a linear manner to 95 mmol C m^{-2} until the following dawn. The sucrose

concentration in the Low treatment was slightly higher immediately after dawn than that in the High treatment, but it was significantly lower from 4 h after dawn to the following dawn. It peaked at 130 mmol C m^{-2} and then decreased to 70 mmol C m^{-2}. The glucose concentration in the High treatment was significantly higher than that in the Low treatment at some time points; however, it remained almost constant throughout the day at approximately 25 mmol C m^{-2} in both treatments. The fructose concentration remained nearly constant throughout the day (at approximately 80 mmol C m^{-2}) in both treatments and did not differ significantly between the treatments. The starch concentration in the High treatment started to increase a little behind the increase in sucrose concentration. It increased from approximately 25 mmol C m^{-2} to 135 mmol C m^{-2}, peaked at dusk, and then decreased in a linear manner to approximately 50 mmol C m^{-2} until the following dawn. Although the starch concentration in the Low treatment was slightly higher immediately after dawn than that in the High treatment, similar to sucrose, it was significantly lower than that in the High treatment from 6 h after dawn to the following dawn, with the daily maximum being only about 40 mmol C m^{-2}, less than a third of that in the High treatment.

We previously found similarities in the dynamics of carbohydrates during three different cultivation periods under field conditions (Nakai et al. 2022). Specifically, sucrose concentration began to increase at dawn and peaked 2–4 h before dusk, glucose and fructose concentrations both remained constant throughout the day, and starch concentration increased significantly after sucrose concentration did and peaked at dusk. These current findings are consistent with our previously reported findings (Nakai et al. 2022), except for the trends in starch concentrations in the Low treatment. The concentration of total carbohydrates (sucrose, glucose, fructose, and starch) in the High treatment was markedly higher than that in the Low treatment, which proves that photosynthesis occurred more actively in the High treatment than in the Low treatment. Thus, the present study results suggest that photosynthetically fixed carbon was allocated to both sucrose and starch under high photosynthetic activity conditions, whereas it was preferentially allocated to sucrose than starch under low photosynthetic activity conditions. Conversely, glucose and fructose showed stable dynamics throughout the day, suggesting that their roles as osmolytes may be maintained even if photosynthetic activity fluctuates.

Relationships between sucrose and starch concentrations

We investigated the relationships between sucrose and starch concentrations in the daytime and nighttime (Fig. 3) based on the diurnal changes in leaf carbohydrate concentrations (Fig. 2). The phased increase in starch concentration in the High treatment depended on the sucrose concentration during the day; it increased gradually from approximately 25 to 50 mmol C m^{-2} until the sucrose concentration reached about 150 mmol C m^{-2}, then increased rapidly to approximately 135 mmol C m^{-2} until 2 h before dusk. In the Low treatment, sucrose concentration did not reach 150 mmol C m^{-2}, and the starch concentration increased gradually from approximately 25 to 50 mmol C m^{-2}, following a similar pattern to that in the High treatment. The starch concentration in the nighttime decreased linearly with sucrose concentration in both treatments. These results are consistent with our previously reported results (Nakai et al. 2022) that suggested a biphasic increase in starch concentration during the daytime and the effects of sucrose concentration on the carbon allocation in strawberry leaves. However, as mentioned above, our previously reported findings were obtained under field conditions over three different cultivation periods; therefore, the previous study could not exclude

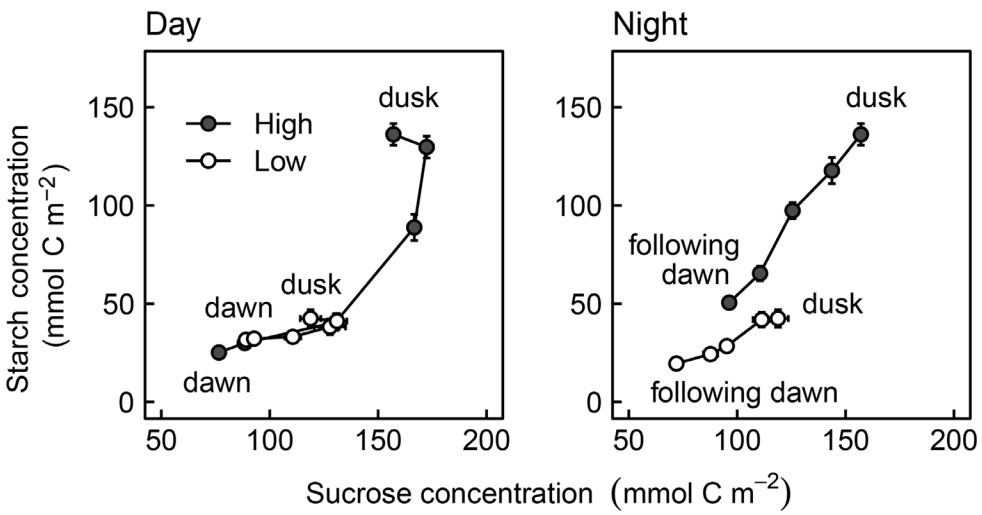

Fig. 3 Relationships between sucrose and starch concentrations in two treatments: high photosynthetic activity treatment (High; closed circles) and low photosynthetic activity treatment (Low; opened circles). Plots are connected in order of time per treatment. Error bars represent the standard error of the mean values ($n = 4$)

the possibility that other seasonal factors, in addition to photosynthetic activity, may have affected the relationship between sucrose and starch concentrations when considering the regulation of carbon allocation. In the present study, the difference in photosynthetic activity during the same cultivation period confirmed the dynamics of sucrose and starch. The current findings provide additional evidence that photosynthetically fixed carbon is allocated preferentially to sucrose than starch when leaf sucrose concentration is less than the storage capacity of 150 mmol C m^{-2}, whereas it is allocated to starch as an overflow product when photosynthesis is active and sucrose concentration exceeds the storage capacity.

As previously mentioned, the mechanisms underlying the regulation of carbon allocation are not fully clarified. Carbon allocation between sucrose and starch is directly linked to the distribution of triose phosphate between the chloroplasts and cytosol. Triose phosphates and their precursor, 3-phosphoglycerate (3-PGA), are transported from the chloroplast into the cytosol in exchange of inorganic phosphates (Pi) in the cytosol via the triose phosphate/phosphate translocator (TPT). Regarding the overflow hypothesis, starch synthesis is activated through the following mechanisms: when photosynthetic activity is high and sucrose and/or its intermediates accumulate in the cytosol, sucrose phosphate synthase (SPS) or fructose-1,6-bisphosphatase (key enzymes in the sucrose synthesis pathway in the cytosol) is inactivated (Stitt 1990; Huber and Huber 1996). This results in the inhibition of the entire pathway of sucrose synthesis and a decrease in the release of Pi in the cytosol. As the transport activity of TPT depends on the availability of Pi in the cytosol, the exchange of triose phosphates/3-PGA and Pi is inhibited, leading to the starvation of Pi and accumulation of triose phosphates and 3-PGA in the chloroplast. Because ADP-glucose pyrophosphorylase, the first enzyme in the starch synthesis pathway, is activated by 3-PGA and inhibited by Pi (Ballicora et al. 2004), starch synthesis is consequently activated. These mechanisms can explain the results of the present study that starch concentration rapidly increased when sucrose accumulated under conditions of high photosynthetic activity. However, the feedback regulation of sucrose described above does not fully explain the mechanism of carbon allocation (Smith and Stitt 2007), and other mechanisms such as circadian control can contribute to the regulation of carbon allocation (Graf et al. 2010; Graf and Smith 2011; Sulpice et al. 2014). This study aimed to investigate carbon allocation based on carbohydrate dynamics. However, the analysis of enzyme activity and the concentrations of intermediates involved in these mechanisms would be useful to further elucidate the regulation of carbon allocation in strawberry leaves.

Conclusions

In summary, we investigated the relationships between sucrose and starch dynamics in strawberry leaves under a controlled environment with high and low photosynthetic activity. Our findings strongly suggest that in the regulation of carbon allocation in source leaves, starch serves as an overflow product at a sucrose concentration of 150 mmol C m^{-2} established as a threshold. Although the relationship between sucrose and starch likely varies depending on the strawberry variety and/or cultivation conditions, the fact that both experiments—one in the field environment with different day lengths according to the season and the other in a controlled environment with same day lengths but with two different photosynthetic activities—showed consistent results strongly suggests that the relationship is an inherent characteristic of strawberry plants.

Further research is required to understand the molecular mechanism of carbon allocation; however, we believe that the present study encourages the translation of fundamental research on model plants to that on agricultural crops and contributes to a better understanding of strawberry productivity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10725-023-01042-9.

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Author contributions Hiromi Nakai: Conceptualization, Investigation, Methodology, Writing - Original Draft. Daisuke Yasutake: Supervision, Conceptualization, Writing – review & editing, Funding acquisition, Project administration. Kota Hidaka: Resources, Writing – review & editing. Koichi Nomura: Resources, Writing – review & editing. Toshihiko Eguchi: Resources, Writing – review & editing. Gaku Yokoyama: Supervision, Writing – review & editing. Tomoyoshi Hirota: Supervision, Writing – review & editing.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statements & Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

References

Annunziata MG, Apelt F, Carillo P, Krause U, Feil R, Koehl K, Lunn JE, Stitt M (2018) Response of *Arabidopsis* primary metabolism and circadian clock to low night temperature in a natural light environment. J Exp Bot 69:4881–4895. https://doi.org/10.1093/jxb/ery276

Ballicora MA, Iglesias AA, Preiss J (2004) ADP-glucose pyrophosphorylase: a regulatory enzyme for plant starch synthesis. Photosynth Res 79:1–24. https://doi.org/10.1023/B:PRES.0000011916.67519.58

R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed 27 February 2023

Cseke C, Balogh A, Wong JH, Buchanan BB, Stitt M, Herzog B, Heldt HW (1984) Fructose 2,6-bisphosphate: a regulator of carbon processing in leaves. Trends Biochem Sci 9:533–535. https://doi.org/10.1016/0968-0004(84)90284-6

Dong S, Beckles DM (2019) Dynamic changes in the starch-sugar interconversion within plant source and sink tissues promote a better abiotic stress response. J Plant Physiol 234–235:80–93. https://doi.org/10.1016/j.jplph.2019.01.007

Duursma RA (2015) Plantecophys - An R package for analysing and modelling leaf gas exchange data. PLoS ONE 10:1–13. https://doi.org/10.1371/journal.pone.0143346

Farquhar GD, Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78–90

Geiger DR, Servaites JC (1994) Diurnal regulation of photosynthetic carbon metabolism in C_3 plants. Annu Rev Plant Physiol Plant Mol Biol 45:235–256. https://doi.org/10.1146/annurev.pp.45.060194.001315

Gibon Y, Pyl ET, Sulpice R, Lunn JE, HÖhne M, GÜnther M, Stitt M (2009) Adjustment of growth, starch turnover, protein content and central metabolism to a decrease of the carbon supply when *Arabidopsis* is grown in very short photoperiods. Plant Cell Environ 32:859–874. https://doi.org/10.1111/j.1365-3040.2009.01965.x

Gordon AJ, Ryle GJA, Webb G (1980) The relationship between sucrose and starch during "dark" export from leaves of uniculm barley. J Exp Bot 31:845–850. https://doi.org/10.1093/jxb/31.3.845

Graf A, Smith AM (2011) Starch and the clock: the dark side of plant productivity. Trends Plant Sci 16:169–175. https://doi.org/10.1016/j.tplants.2010.12.003

Graf A, Schlereth A, Stitt M, Smith AM (2010) Circadian control of carbohydrate availability for growth in *Arabidopsis* plants at night. Proc Natl Acad Sci U S A 107:9458–9463. https://doi.org/10.1073/pnas.0914299107

Huber SC, Huber JL (1996) Role and regulation of sucrose-phosphate synthase in higher plants. Annu Rev Plant Physiol Plant Mol Biol 47:431–444. https://doi.org/10.1146/annurev.arplant.47.1.431

Kimura K, Yasutake D, Koikawa K, Kitano M (2020) Spatio-temporal variability of leaf photosynthesis and its linkage with microclimates across an environment-controlled greenhouse. Biosyst Eng 195:97–115. https://doi.org/10.1016/j.biosystemseng.2020.05.003

Lunn JE, Hatch MD (1995) Primary partitioning and storage of photosynthate in sucrose and starch in leaves of C_4 plants. Planta 197:5000

MacNeill GJ, Mehrpouyan S, Minow MAA, Patterson JA, Tetlow IJ, Emes MJ (2017) Starch as a source, starch as a sink: the bifunctional role of starch in carbon allocation. J Exp Bot 68:4433–4453. https://doi.org/10.1093/jxb/erx291

McClain AM, Sharkey TD (2019) Triose phosphate utilization and beyond: from photosynthesis to end product synthesis. J Exp Bot 70:1755–1766. https://doi.org/10.1093/jxb/erz058

Medlyn BE, Duursma RA, Eamus D, Ellsworth DS, Prentice IC, Barton CVM, Crous KY, De Angelis P, Freeman M, Wingate L (2011) Reconciling the optimal and empirical approaches to modelling stomatal conductance. Glob Chang Biol 17:2134–2144. https://doi.org/10.1111/j.1365-2486.2010.02375.x

Mengin V, Pyl ET, Moraes TA, Sulpice R, Krohn N, Encke B, Stitt M (2017) Photosynthate partitioning to starch in *Arabidopsis thaliana* is insensitive to light intensity but sensitive to photoperiod due to a restriction on growth in the light in short photoperiods. Plant Cell Environ 40:2608–2627. https://doi.org/10.1111/pce.13000

Nakai H, Yasutake D, Kimura K, I K, Hidaka K, Eguchi T, Hirota T, Okayasu T, Ozaki Y, Kitano M (2022) Dynamics of carbon export from leaves as translocation affected by the coordination of carbohydrate availability in field strawberry. Environ Exp Bot 196:104806. https://doi.org/10.1016/j.envexpbot.2022.104806

Nishizawa T (1994) Comparison of carbohydrate partitioning patterns between fruiting and deflorated June-bearing strawberry plants. J Japan Soc Hort Sci 62:795–800

Rösti S, Fahy B, Denyer K (2007) A mutant of rice lacking the leaf large subunit of ADP-glucose pyrophosphorylase has drastically reduced leaf starch content but grows normally. Funct Plant Biol 34:480–489. https://doi.org/10.1071/FP06257

Smith AM, Stitt M (2007) Coordination of carbon supply and plant growth. Plant Cell Environ 30:1126–1149. https://doi.org/10.1111/j.1365-3040.2007.01708.x

Smith AM, Zeeman SC (2020) Starch: a flexible, adaptable carbon store coupled to plant growth. Annu Rev Plant Biol 71:217–245. https://doi.org/10.1146/annurev-arplant-050718-100241

Stitt M (1990) Fructose-2,6-bisphosphate as a regulatory molecule in plants. Annu Rev Plant Physiol Plant Mol Biol 41:153–185. https://doi.org/10.1146/annurev.pp.41.060190.001101

Stitt M, Zeeman SC (2012) Starch turnover: pathways, regulation and role in growth. Curr Opin Plant Biol 15:282–292. https://doi.org/10.1016/j.pbi.2012.03.016

Stitt M, Lunn J, Usadel B (2010) *Arabidopsis* and primary photosynthetic metabolism - more than the icing on the cake. Plant J 61:1067–1091. https://doi.org/10.1111/j.1365-313X.2010.04142.x

Streb S, Zeeman SC (2012) Starch metabolism in *Arabidopsis*. Arab B 10:e0160. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3527087/

Sulpice R, Flis A, Ivakov AA, Apelt F, Krohn N, Encke B, Abel C, Feil R, Lunn JE, Stitt M (2014) *Arabidopsis* coordinates the diurnal regulation of carbon allocation and growth across a wide range

of Photoperiods. Mol Plant 7:137–155. https://doi.org/10.1093/mp/sst127
Taiz L, Zeiger E, Moller IM, Murphy A (2018) Plant Physiology and Development, 6th edn. Sinauer Associates, Sunderland