Yield and Photosynthesis Related to Growth Forms of Two Strawberry Cultivars in a Plant Factory with Artificial Lighting

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Abstract. Appropriate growth forms for strawberry production in a plant factory with artificial lighting (PFAL), which is a recently developed production system, remain undetermined. Improving strawberry productivity in a PFAL requires insights into the interplay between production characteristics (growth and photosynthesis) and growth forms, such as plant height and leaf area (LA), which are major determinants of crop yield. Growth status, yield, and photosynthetic characteristics of the two cultivars of strawberries (Fragaria ×ananassa Duch. Tochiotome and Koiminori) with different growth forms were examined. 'Koiminori' exhibited a 1.9-fold higher yield and a 2.0-fold greater total dry weight of respective organs compared with 'Tochiotome'. The single-plant photosynthetic rate (A') exhibited an index for both cultivars, was 2.2-times higher for Koiminori than for Tochiotome. The photosynthetic rates of a single leaf (A_L) and A were also analyzed as important factors that influence the A'. The A_L for 'Koiminori' surpassed that of 'Tochiotome' by 1.4 times. This was attributed to the elevated photosynthetic photon flux density received by the upper leaves of Koiminori, which is a consequence of its higher plant height in proximity to the light source. Evaluation of four photosynthetic capacities, maximum rate of carboxylation, maximum rate of electron transport, photosynthetic rate under saturating light, and light utilization efficiency, which are potential factors that affect A_L, revealed no differences in these capacities between cultivars. 'Koiminori' exhibited a significantly larger LA (2.3- to 3.1-times) than 'Tochiotome', indicating that the former's higher A_L resulted mainly from its higher A_L and larger LA. Thus, strawberry production in a PFAL can be improved by growing cultivars with growth forms such as higher plant height and larger LA.

A plant factory with artificial lighting (PFAL) is expected to serve as a stable food production system and is designed to consistently and systematically yield crops throughout the year. The PFAL overcomes the limitations imposed by seasonal variations and climate-related disasters (e.g., typhoons or sudden heavy rainfall) on crop production because the entire environment surrounding the crop can be protected and controlled (Beacham et al. 2019). Additionally, proper management can enable pesticide-free cultivation of crops in closed crop production, thus effectively discouraging pathogen invasion (Kozai 2021). Furthermore, the PFAL enables multitier cultivation, enhances land use efficiency, and fosters urban agriculture, consequently lowering transport costs (Avgoustaki and Xydis 2020).

Despite its advantages, the PFAL also has disadvantages, such as the high cost of cultivation (Kalantari et al. 2018). In particular, the costs of controlling environmental conditions to optimize crop growth are the major problem. Approximately 25% of operational expenses are attributed to electricity costs, with lighting constituting 75% of this expenditure (Kozai 2021). Therefore, to reduce electricity costs, the traditional crops cultivated in the PFAL are typically leafy vegetables, which can be grown at relatively low light levels (Armanda et al. 2019). Additionally, they can be easily applied to multitier cultivation because of their low plant height. However, addressing the challenge of high electricity costs requires the identification of more profitable crops. Therefore, strawberry cultivation in the PFAL is attracting attention because of its high market value (Wortman et al. 2016). Compared with other fruits and vegetables (e.g., tomatoes and cucumbers), strawberries can be grown under relatively low light intensity (Maeda and Ito 2020).

However, the implementation of strawberry cultivation in a PFAL is limited, and basic long-term data such as growth status [e.g., plant height and leaf area (LA)] and yield are lacking. Consequently, assessing the viability of strawberries as suitable PFAL crops and adopting a scientific approach for improved optimization are hindered. This research gap highlights the need to explore the growth status and yield characteristics of strawberries in a PFAL to obtain and accumulate data regarding their long-term variation. The growth status and yield depend on the canopy photosynthetic rate (Nomura et al. 2020; Parry et al. 2011), which depends on the LA and photosynthetic rate of a single leaf (Kaneko et al. 2022). Furthermore, the photosynthetic rate of a single leaf also depends on the maximum rate of carboxylation (V_cmax), maximum rate of electron transport (U_max), photosynthetic rate under saturating light (A_max), and light utilization efficiency (Φ), which are important parameters for examining the biochemical and light response processes of photosynthesis. Therefore, to assess crop growth and yields, the photosynthetic rate and capacity of a canopy and a single leaf should be evaluated.

Strawberry cultivation in a PFAL requires a larger space above the cultivation bed for crop management (removing leaves and fruits and harvesting) during long-term cultivation compared with that needed for leafy vegetable cultivation. Under such space conditions, cultivars with higher plant heights should be advantageous because they receive higher light intensity because of the attenuation of
the intensity of artificial light with increasing distance from the light source. Thus, plant height should be a main factor that determines the crop yield in the PFAL. Investigations of the growth status, yield, and photosynthetic characteristics of strawberry cultivars and the incorporation of growth forms (plant height and LA) into the PFAL are required.

The objective of this study was to clarify the characteristics of strawberry production (yield, growth, and photosynthesis) in relation to growth forms in the PFAL. In the present study, we measured the growth status as the growth forms, yields, and photosynthetic characteristics of strawberry plants. We measured the photosynthetic capacities ($V_{\text{emax}}$, $A_{\text{max}}$, $\Phi$) of two cultivars with different growth forms throughout the long-term cultivation period.

Materials and Methods

Plant materials and cultivation. Two strawberry cultivars (Fragaria *x* ananassa Duch. “Tochiotome” and “Koiminori”) were grown in a PFAL located at the Joshima Factory of OREC Co., Ltd. (Kurume City, Fukuoka, Japan; 33°25’8”N, 130°43.2’E). On 22 Feb 2021, young plants were transplanted to cultivation beds [1.22 m (length) × 0.34 m (width) × 0.17 m (height)] filled with porous grains of diatomaceous earth (ISOLITE CG; Isolate Insulating Products Co., Ltd., Osaka, Japan), with 0.2 m between plants and 0.15 m between rows to ensure growth conditions (Fig. 1). The distance from the top of the cultivation beds to the light source was 0.3 m for daily cultivation management, such as removing leaves and fruits and harvesting (Fig. 2). A nutrient solution (OAT House No. 1; OAT House No. 2; OAT House No. 5 = 60:40:1; OAT Agro Co., Ltd., Tokyo, Japan) with electrical conductivity (EC) of 0.6 dS m⁻¹ was supplied at a rate of 385 mL per day per plant every 30 min from 8:00 HR to 16:00 HR. Four light-emitting diode (LED) lamps (LT8018AP0104; 18W, Hansen Japan Co., Ltd., Osaka, Japan), with a fixed 30 cm above the cultivation bed. Figure 3 presents the spectral distributions of the lamps. The air temperature was maintained at 25°C/20°C during the photoperiod and dark period from 23 Jun 2021 to 31 Aug 2021, and at 22°C/17°C from 1 Sep 2021 to 24 Jan 2022. Although the relative humidity (RH) was controlled at 80% ± 5%, the CO₂ concentration was controlled at 800 µmol mol⁻¹ during the photoperiod without a control dark period.

Environmental measurements. To investigate the cultivation environment, we introduced sensors near each strawberry plant cultivar. The photosynthetic photon flux density (PPFD) was measured using photon quantum sensors (PAR-02D; Prede Co., Ltd., Tokyo, Japan) placed at a point corresponding to plant height and changed by plant growth during the experiment. The air temperature and RH were measured using a temperature and humidity sensor (HMP110; VAIASA Co., Ltd., Tokyo, Japan) inside a forced ventilator (RSSH01A1203; CSE Inc., Sapporo, Japan), and the air CO₂ concentration was measured using a CO₂ sensor (GMP222; VAIASA Co., Ltd., Tokyo, Japan). Data were recorded at 5-min intervals using a data logger (GL240; GRAPHTEC Co., Ltd., Kanagawa, Japan).

Measurements of plant height, leaf area, soil plant analysis development, and fruit yield. The plant height and leaf area (LA) of single plants were measured monthly. The leaf area per leaf was estimated by measuring the leaflet length ($L_{1}$) and leaflet width ($W_{1}$) using Eq. [1]. This equation was obtained based on the relationship between the products of the $L_{1}$ multiplied by the $W_{1}$ and the leaf areas of individual leaves, as described by Hidaka et al. (2013).

$$LA = a \ (L_{1} \times W_{1}) + b \ [1]$$

where $a$ and $b$ represent constant values for the two cultivars ($a = 1.647$ and $b = 11.666$ for Tochiotome; $a = 2.157$ and $b = 3.572$ for Koiminori). The coefficients of determination were 0.7928 for Tochiotome and 0.8924 for Koiminori.

The soil plant analysis development (SPAD) value that correlated with the leaf chlorophyll concentration was measured monthly using a chlorophyll meter (SPAD-502; Konica Minolta, Inc., Tokyo, Japan). Fully ripe fruits were harvested daily from Jul 2021 to Dec 2021 to determine the yield of the cultivars.

Measurements of dry matter in strawberries. Removed leaves were dried for 72 h at 80°C in a circulation dryer and weighed every month. On the last day of the experiment (24 Jan 2022), eight Tochiotome and Koiminori plants were harvested and separated into their respective organs (leaves, fruits, crowns, and roots). Each part was dried for 72 h at 80°C in a circulation dryer and weighed.

Photosynthetic characteristics. The photosynthetic rates of a single plant ($A_{p}$) of Tochiotome and Koiminori were measured using a closed chamber measuring 25, 17, and 24 cm in height, width, and length,
respectively. The chamber system mainly consisted of a closed PVC chamber, an infrared CO$_2$ gas analyzer (GMP343; VAISALA Co., Ltd., Tokyo, Japan), and an air pump (CM-15-24; Enomoto Micro Pump Mfg. Co., Ltd., Tokyo, Japan). The system used to measure the time change in the CO$_2$ concentration and the method used to calculate $A_P$ (CO$_2$ exchange rate of a single plant) followed those described by Ono et al. (2022). The $A_P$ was calculated using the following equation:

$$A_P = V \times \frac{\Delta C}{T} \quad [2]$$

where $V$ represents the volume of the chamber (m$^3$), $T$ represents the elapsed time since the chamber (s) was closed, and $C$ represents the CO$_2$ density ($\mu$mol-m$^{-3}$). $C$ was calculated using the following equation:

$$C = [\text{CO}_2] \times \frac{1}{0.0224 \times \frac{273 + T}{273}} \quad [3]$$

where [CO$_2$] represents the CO$_2$ concentration ($\mu$mol-m$^{-1}$) and $T$ represents the air temperature inside the chamber.

The single-leaf photosynthetic rates of Tochiotome and Koiminori ($A_1$) were measured using a portable open gas exchange system (LI-6400XT; LI-COR, Inc., Lincoln, NE, USA) with a transparent top chamber (standard leaf chamber; LI-COR) on 24 Nov 2021 and 28 Dec 2021. The environmental conditions controlled inside the chamber were as follows: air temperature, RH, and CO$_2$ concentration of ~25 °C, 50% to 60%, and 800 µmol mol$^{-1}$, respectively.

To evaluate the leaf photosynthetic ability of Tochiotome and Koiminori, the maximum rate of carboxylation ($V_{c_{\text{max}}}$) and maximum rate of electron transport ($J_{\text{max}}$) were estimated using the $A$-$C$ curve method or one-point method (De Kauwe et al. 2016) using the LI-6400XT with an LED light source chamber. Then, $A_{\text{max}}$ and $\Phi$ were evaluated.

$$A = \frac{0.4PPFD + A_{\text{max}} - \sqrt{(0.4PPFD + A_{\text{max}})^2 - 4PPFDs_{\text{max}}}}{29} \quad [4]$$

where $\theta$ represents the convexity of the $A$-PPFD curve.

**Statistical analysis.** Experimental data obtained from the plant height ($n = 5$), LA ($n = 5$), SPAD ($n = 5$), fruit yield ($n = 4$), dry weight (DW) of respective organs ($n = 8$), PPFD ($n = 16$), $A_P$ ($n = 3$, $A_L$ ($n = 16$), $V_{c_{\text{max}}}$ ($n = 19$), $J_{\text{max}}$ ($n = 19$), $A_{\text{max}}$ ($n = 5$), and $\Phi$ ($n = 5$) were subjected to the Student $t$ test using R software (version 1.4.1717; R Development Core Team, Vienna Austria). $P < 0.05$ was considered statistically significant.

**Results**

**Cultivation environments.** Figure 4 shows the daily changes in the integrated PPFD ($I$) (Fig. 1A), average air temperature ($T_A$) (Fig. 1B), average RH (Fig. 1C), and average CO$_2$ concentration ($C_A$) (Fig. 1D) for the two strawberry cultivars (Tochiotome and Koiminori) during the entire experimental period from 25 Jun 2021 to 23 Jan 2022. The $I$ values observed at the tops of the canopies of Tochiotome and Koiminori were ~10 and 15 mol m$^{-2}$ d$^{-1}$ from Jun 2021 to Oct 2021 and ~13 and 16 mol m$^{-2}$ d$^{-1}$ from Nov 2021 to Jan 2022, respectively. The intensity of the artificial light decreased with the increasing distance from the light source. Therefore, this difference between Tochiotome and Koiminori can be attributed to the installation of photon quantum sensors in accordance with each plant height, but not to the changing light intensity of the light source. Tochiotome and Koiminori had similar $T_A$, RH, and $C_A$ values; $T_S$ values were approximately 24 °C from Jun 2021 to Aug 2021 and approximately 22 °C from Sep 2021 to Jan 2022, the RH was approximately 80% ± 5% throughout the experiment, and $C_A$ gradually increased from June to September and then were maintained at approximately 700 ± 50 µmol mol$^{-1}$.

**Plant growth dynamics.** Figure 5 shows the time change for plant height, LA, and SPAD values of the plants of the two strawberry cultivars (Tochiotome and Koiminori) during the entire experimental period. The plant heights of Tochiotome and Koiminori were approximately 151.2 ± 2.2 cm and approximately 22.9 ± 2.9 cm, respectively. The LA of Tochiotome varied from 500 to 1000 cm$^2$/plant throughout the experimental period, and that of Koiminori varied from 500 to 1000 cm$^2$/plant during the first 2 months; thereafter, they varied from 1500 to 3000 cm$^2$/plant. The SPAD values varied from 50 to 55 and from 43 to 52 for Tochiotome and Koiminori, respectively. Koiminori exhibited a significantly higher plant height and LA than those of Tochiotome during the experimental period, except for those during the first 2 months. Koiminori exhibited markedly lower SPAD values (which are correlated with leaf chlorophyll contents) than Tochiotome throughout the experimental period.

**Yield and dry weights of the respective organs of crops.** Figure 6 shows the monthly accumulations of Tochiotome and Koiminori fruit yields per plant. Koiminori exhibited a significantly higher yield (321.5 g/plant) than Tochiotome (168.7 g/plant) throughout the experimental period. Table 1 shows the DW of the respective plant organs (removed leaf, leaf, fruit, crown, and root) and their ratios to those of the entire plant body of the two

![Fig. 4. Daily changes in integrated photosynthetic photon flux density ($I$) (A), average air temperature ($T_A$) (B), average relative humidity (RH) (C), and average CO$_2$ concentration ($C_A$) (D) as a function of time for the two strawberry cultivars (Tochiotome and Koiminori) during the entire experimental period from 25 Jun 2021 to 23 Jan 2022. The solid and dashed lines represent the data of Tochiotome and Koiminori, respectively.](image-url)
strawberry cultivars (Tochiotome and Koiminori) at the end of the cultivation experiment. The value for Koiminori was approximately twice that of Tochiotome. No differences in the relative DWs of the respective organs were observed between Tochiotome and Koiminori.

Photosynthetic characteristics. Figure 7 shows the PPFD on the surface of upper leaves of Tochiotome and Koiminori, Ap and A1. Koiminori had a higher PPFD (400 μmol·m⁻²·s⁻¹) than Tochiotome (300 μmol·m⁻²·s⁻¹). This difference occurred because Koiminori had greater plant heights than Tochiotome, and its leaves in the upper part of the canopy were closer to the light source than those of Tochiotome. Koiminori also had significantly higher Ap and A1 than Tochiotome. Koiminori had 1.4-times higher PPFD and A1 values and 2.2-times higher Ap values than Tochiotome. The differences in A1 and Ap were attributed to differences in PPFD because both parameters are known to increase until the PPFD reaches ~500 μmol·m⁻²·s⁻¹ in strawberries (Mochizuki et al. 2019; Trong et al. 2021). Table 2 shows the V_{cmax}, J_{max}, A_{max} and Φ of Tochiotome and Koiminori; no significant differences in the parameters for either cultivars were observed.

**Discussion**

Factors affecting the yield of PFAL. The yield and DW of plant organs are often correlated with the total photosynthetic content during the cultivation period (Heuvelink 2005; Yoneda et al. 2020). During this study, the yield of Koiminori was 1.9-times higher than that of Tochiotome at the end of cultivation (Fig. 6), and the total DW of the plant organs of Koiminori was twice that of Tochiotome (Table 1).

The Ap of Koiminori was 2.2-times higher than that of Tochiotome (Fig. 7), indicating that the differences in the yields and dry weights between cultivars are attributable to differences in their Ap. The Ap should be strongly affected by the A1 and LA. The A1 was 1.4-times higher for Koiminori than for Tochiotome (Fig. 7). The difference in A1 between cultivars can be attributed to the difference in the PPFD at the upper position of the canopy (Tochiotome, 280 μmol·m⁻²·s⁻¹; Koiminori, 400 μmol·m⁻²·s⁻¹) because light intensity is the major factor that amplifies photosynthesis (Choi et al. 2016; Hidaka et al. 2013; Iwao et al. 2021). This difference in PPFD should be attributable to the difference in their plant height because that of Koiminori was 1.2- to 1.8-times higher than that of Tochiotome, and the upper leaves of its canopy received more light because they were closer to the light source during the cultivation (Figs. 5 and 7).

Other factors that strongly affect A1 include V_{cmax}, J_{max}, A_{max}, and Φ, which represent the photosynthetic capacity based on the biochemical and light response processes of photosynthesis (Chen et al. 2014; Wilson et al. 2000). During this study, four parameters, V_{cmax}, J_{max}, A_{max}, and Φ, were examined to investigate whether differences in the photosynthetic capacities of a single leaf existed between cultivars; however, no significant differences between cultivars was observed in these parameters (Table 2), indicating that photosynthetic capacities did not induce a difference in A1 between cultivars. Therefore, the difference in PPFD caused by plant height should cause a difference in the A1 in the PFAL.

For Koiminori, a higher LA should contribute to a higher A1 because it had 2.3-times to 3.1-times higher LA than Tochiotome, except for that during the first 2 months (Fig. 5). Furthermore, a positive feedback loop seemed to exist between photosynthesis and growth; the leaf growth should induce increased Ap, which is expected to accelerate leaf growth (Nomura et al. 2021). Thus, growth forms (plant height and LA) of the two cultivars are expected to result in differences in their yield based on the DW, A1, and A1 of the respective cultivars within the PFAL. Therefore, we concluded that a higher plant height suited for PFAL cultivation, the applicability of these results to other cultivars within the PFAL is not guaranteed because only two cultivars were considered during this study. Because some strawberry cultivars may have different levels of photosynthetic capacity.

**Table 1. Dry weight (DW) of the respective plant organs (leaf, removed leaf, fruit, harvested fruit, crown, and root) and its ratio to that of the entire plant body (values in parentheses) for the two strawberry cultivars (Tochiotome and Koiminori) at the end of the cultivation experiment (24 Jan 2022).**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Tochiotome</th>
<th>Koiminori</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf (g)</td>
<td>3.39 ± 1.13 a</td>
<td>9.25 ± 4.25 b</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(9)</td>
</tr>
<tr>
<td>Removed leaf (g)</td>
<td>16.05</td>
<td>32.07</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>(31)</td>
</tr>
<tr>
<td>Fruit (g)</td>
<td>0.86 ± 0.56 a</td>
<td>1.98 ± 1.26 a</td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(2)</td>
</tr>
<tr>
<td>Harvested fruit (g)</td>
<td>21.07 ± 1.26 a</td>
<td>39.35 ± 3.12 b</td>
</tr>
<tr>
<td></td>
<td>(40)</td>
<td>(38)</td>
</tr>
<tr>
<td>Crown (g)</td>
<td>4.14 ± 1.87 a</td>
<td>10.48 ± 2.07 b</td>
</tr>
<tr>
<td></td>
<td>(14)</td>
<td>(10)</td>
</tr>
<tr>
<td>Root (g)</td>
<td>7.16 ± 4.34 a</td>
<td>10.86 ± 6.25 a</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td>Total (g)</td>
<td>52.66 ± 5.86 a</td>
<td>103.98 ± 10.24 b</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(100)</td>
</tr>
</tbody>
</table>

Values are presented as the means ± SD (n = 8). Different letters indicate significant differences in DW between cultivars (P < 0.05, Student’s t test).
Table 2. Maximum rate of carboxylation ($V_{cmax}$), maximum rate of electron transport ($J_{max}$), photosynthetic rate at light saturation ($A_{max}$), and initial slope of the $A$-PPFD curve ($\Phi$) for the two strawberry cultivars (Tochiotome and Koiminori).

<table>
<thead>
<tr>
<th>Photosynthetic capacity</th>
<th>Tochiotome</th>
<th>Koiminori</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{cmax}$ (mol m$^{-2}$ s$^{-1}$)</td>
<td>67.67 ± 0.89 a</td>
<td>63.28 ± 0.63 a</td>
</tr>
<tr>
<td>$J_{max}$ (mol m$^{-2}$ s$^{-1}$)</td>
<td>150.33 ± 21.62 a</td>
<td>155.98 ± 24.35 a</td>
</tr>
<tr>
<td>$A_{max}$ (mol m$^{-2}$ s$^{-1}$)</td>
<td>32.76 ± 6.47 a</td>
<td>38.62 ± 6.15 a</td>
</tr>
<tr>
<td>$\Phi$</td>
<td>0.11 ± 0.02 a</td>
<td>0.13 ± 0.01 a</td>
</tr>
</tbody>
</table>

Values represent mean ± SD ($V_{cmax}$, $J_{max}$, $A_{max}$, $\Phi$ n = 19; $A_{max}$, $\Phi$ n = 5). Different letters indicate significant differences between cultivars ($P < 0.05$, Student’s t test).