Possibility of Harvesting June-bearing Strawberries in a Plant Factory with Artificial Light during Summer and Autumn by Re-using Plants Cultivated by Forcing Culture

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INTRODUCTION

In Japan, June-bearing strawberry cultivars are commercially produced from November to May in forcing greenhouses (Yoshida and Nishimoto, 2020), and June to October is off-season due to their physiological characteristics (Yamasaki, 2013; Yamazaki and Yano, 2016). However, fresh strawberry fruits are in demand all year round for eating raw or topping cakes (Yanagi, 2017), and approximately, 3,000 tons are imported annually from the United States to fill the supply gap (Tokyo Customs, 2014; Ministry of Agriculture, Forestry and Fisheries, 2019). As the appearance and taste of the imported fruits are not highly appreciated in the Japanese market, in addition to concerns on pesticide application and post-harvest treatments abroad, domestically produced strawberries are preferred (Imada, 2007). Therefore, everbearing strawberries are planted in cold areas for off-seasons to increase the production of domestic strawberries in summer and autumn (Yamazaki, 2015; Ohta and Yasuba, 2019). On the other hand, as confectioneries claim that everbearing strawberries have a poorer taste than that of June-bearing strawberries (Shibuya, 2010; Hamano et al., 2020), they want a year-round stable supply of June-bearing strawberries.

In the plant factory with artificial light (PFAL), leafy vegetables such as lettuce grow rapidly and yield a year-round high under low-intensity fluorescent and LED lights, with little to no pesticide application (Yoshida et al., 2016), and fresh leafy vegetable production in PFALs is increasing (Goto, 2012). However, fruits, including strawberries, require stronger light intensity to facilitate photosynthesis (Shimizu et al., 2011; Maeda et al., 2016; Furuyama et al., 2017). In addition, the running cost of PFALs for more than 2 months of nursery period will increase, with no strawberry production (Fushihara, 2005). The most important difference between fruit and leafy vegetable cultivation in PFALs is that, the former needs modified day length and temperature in each stage of development for fruit production (Sønsteby and Heide, 2006; Hytönen and Kurokura, 2020). Off-season strawberry production in PFALs is uncommon because of insufficient fruit yield to meet the operational costs (Yoshida et al., 2013). In recent years, PFALs have been developed for

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strawberry cultivation, producing forced strawberries under natural day length conditions in a greenhouse from November to May. Thus, if productive strawberries are transferred from a greenhouse to a PFAL before being exposed to long day and high temperature conditions that inhibit flower bud differentiation, continuous production of June-bearing strawberries may be realized under proper environmental control.

Integrating the two cultivation methods for June-bearing strawberry plants, in greenhouse and PFAL, will lead to a year-round supply of preferred-quality fruits in the market, with limited use of PFALs in summer and autumn when forced culture does not work on mature June-bearing cultivars. This combination will contribute to reduce costs, eliminating the nursery period of strawberries grown in a PFAL.

In this study, three experiments were conducted by transferring strawberries to a PFAL with the aim of improving productivity during after May when strawberry fruit production is difficult in greenhouse cultivation. In Experiment 1, in order to clarify whether summer-autumn production is possible if the plants used in forcing cultivation are transferred to a PFAL, we compared the fruit yields under the conditions where reproductive growth was induced (low-temperature and short-day treatment) and the conditions where vegetative growth was promoted (medium-temperature and medium-day treatment). Furthermore, in Experiment 2, in order to investigate which strawberry cultivars yield is higher in a PFAL, the fruit yields were compared using the four cultivars (“Akihime,” “Benihoppe,” “Tochiotome,” and “Sachinoka”). In addition, in Experiment 3, in order to clarify whether the fruit yield of “Benihoppe”, which had the highest yield in Experiment 2, would increase further when the light intensity is increased, the fruit yields under the strong and weak light intensity conditions were compared, and the leaf photosynthetic rate was investigated.

MATERIALS AND METHODS

Experiment 1: Fruit yield of strawberry “Benihoppe” from April to August
The June-bearing strawberry cultivar “Benihoppe” was studied. In 2013, strawberry nursery plants were planted in non-woven pots (Root wrap, 18 cm diameter; 3.6 L volume; Hasegawakogyo Co., Ltd., Koyu-gun, Miyazaki, Japan) in a greenhouse and grown by forcing. A mixture of Kanuma pumice, vermiculite, and peat moss in a 1:1:1 volumetric ratio was used as a soil substrate, with 0.4 caustic lime as base fertilizer per pot. The strawberry plants were drip fertirrigated by 50% Otsuka-1/2A Nutrient Solution (OAT Agro Co., Ltd., Chiyoda, Tokyo, Japan), with electric conductivity (EC) of 0.6 dS m⁻¹ before anthesis and EC of 1.2 dS m⁻¹ in harvesting period, with an adjusted pH of 6.0–6.5 and drain rate of approximately 30%. In mid-October, the side windows of the greenhouse were closed for heat retention, and fluorescent lighting was applied from 17:00–21:00 and 1:00–2:00 to break dormancy. Starting from early November, the movable ceiling curtains were drawn in the greenhouse at night, and the heater was used to keep the temperature above 5°C. During the day, the ventilation fan was used when the room temperature exceeded 28°C. Pests were removed by splaying water, and honeybees were introduced for pollination during the flowering period. The temperature of the greenhouse was 7°C to 22°C in April, 18°C to 30°C in June, 23°C to 34°C in August and the day length varied from 12 hours 57 minutes to 14 hours 35 minutes.

On April 11, 2014, some strawberry plants grown in the forcing greenhouse, with all fruits and old leaves removed, leaving inflorescences and eight young leaves, were transferred to two rooms of a PFAL, with two different environmental conditions, one is the low-temperature and short-day treatment (20/10°C [light/dark period]-8-hour day length, LS treatment) and another is the medium-temperature and medium-day treatment (25/15°C [light/dark period]-10-hour day length; MM treatment). June-bearing strawberries carry out flower bud differentiation under low-temperature and short-day conditions (Ito and Saito, 1962; Heide, 1997). On the other hand, a high-temperature (≥30°C) environment maintains the vigor of strawberries (Kadir et al., 2006). Therefore, we used LS treatment as an environment in which inflorescences were continuously formed, and MM treatment as another environment where plant vigor was maintained and flower bud differentiation occurred. In the PFAL, throughout the cultivation period, the LS treatment temperature was 12°C to 21°C, whereas the MM treatment temperature was 17°C to 26°C.

Fluorescent lamps (FFH32EX-NH; Panasonic Co., Kadoma-shi, Osaka, Japan) were used as the light sources for LS and MM treatments; the light intensities (Photosynthetic Photon Flux Density (PPFD)) of the leaf surfaces of June-bearing strawberries were 292.5±12.5 μmol m⁻² s⁻¹ in LS treatment and 301.5±8.5 μmol m⁻² s⁻¹ in MM treatment, with 1.0-m distance between the fluorescent lamp and the cultivation shelf.

In the PFAL, the strawberry plants were fertirrigated with Tank Mix A and Tank Mix B (OAT Agro Co., Ltd.), with EC of 1.0 dS m⁻¹ and pH of 6.0, at a drain rate of approximately 30%. In addition, artificial and bumblebee pollinations were performed. The survey period was from April 11 to August 31, 2014, and three plants were used in each treatment. The fruit yield was measured on harvest using an electronic weighing instrument.

Experiment 2: Differences in fruit yields and flower bud emergence among the four strawberry cultivars
June-bearing strawberry cultivars “Akihime,” “Benihoppe,” “Tochiotome,” and “Sachinoka” were studied. Strawberry plants were transferred to a PFAL in May 2013. From May 10 to July 17, three plants per cultivar were grown under low-temperature and short-day conditions and from July 18 to October 31, plants were grown under medium-temperature and medium-day conditions of Experiment 1. Fruit yields, total number of harvested fruits, and total numbers of inflorescences and flowers recorded were analyzed using Microsoft Excel 2013.

100 (60)
RESULTS

Experiment 1: Fruit yield of strawberry “Benihoppe” from April to August

Changes in the fruit yield of “Benihoppe” grown in a PFAL are shown in Fig. 2. The total yields were 404.5 g/plant in LS treatment, 375.2 g/plant in MM treatment, and 146.7 g/plant in the greenhouse. In the greenhouse, the monthly yield was the highest in May and decreased afterwards to almost zero in August. On the other hand, in LS and MM treatments, strawberry production continued without interruption throughout the entire study period, and the highest monthly yield was recorded in August. There was no difference in yield between LS and MM treatments from April to May, but the yields in LS treatment from June to August were higher than that in MM treatment.

Experiment 2: Differences in fruit yields and flower bud emergence among the four strawberry cultivars

Figure 3 shows differences in fruit yields and flower bud emergence among the four strawberry cultivars grown in a PFAL. From May 10 to July 17, three plants per cultivar were grown under low-temperature and short-day conditions of Experiment 1. Although the continuity of flower bud differentiation was good, some plants showed a decline in growth. Therefore, the cultivation was carried out from July 18 to October 31 under medium-temperature and medium-day conditions to keep plants growth. All cultivars were continuously harvested from July to October. The total fruit yield was the highest in “Benihoppe,” followed by “Akihime,” “Tochiotome,” and “Sachinoka.” The yields of “Benihoppe” and “Akihime” showed a decline in growth. Therefore, the cultivation was carried out from July 18 to October 31 under medium-temperature and medium-day conditions to keep plants growth. All cultivars were continuously harvested from July to October. The total fruit yield was the highest in “Benihoppe,” followed by “Akihime,” “Tochiotome,” and “Sachinoka.” The yields of “Benihoppe” and “Akihime” showed a decline in growth. Therefore, the cultivation was carried out from July 18 to October 31 under medium-temperature and medium-day conditions to keep plants growth. All cultivars were continuously harvested from July to October. The total fruit yield was the highest in “Benihoppe,” followed by “Akihime,” “Tochiotome,” and “Sachinoka.”

Fig. 2 Changes in fruit yields of June-bearing strawberry cultivar “Benihoppe” grown in greenhouse and plant factory with artificial light.

**Fig. 2 Changes in fruit yields of June-bearing strawberry cultivar “Benihoppe” grown in greenhouse and plant factory with artificial light.**

"Different colors and patterns in each column represent monthly contributions to total fruit yields recorded from April 11 through August 31, 2014. The strawberry plants were transferred from a greenhouse to a plant factory with artificial light in April 2014 and placed in the control greenhouse under natural day length conditions that varied from 12 hours 57 minutes to 14 hours 35 minutes and in a plant factory with artificial light with fluorescent lamps for the LS (20/10°C [light/dark period]) 8-hour day length at PPFD 292.5 ± 12.5 μmol m⁻² s⁻¹) and MM (25/15°C [light/dark period]) 10-hour day length at PPFD 301.5 ± 8.5 μmol m⁻² s⁻¹). LS: low-temperature and short-day, MM: medium-temperature and medium-day.
were significantly higher than those of “Tochiotome” and “Sachinoka” (Fig. 3A). The number of fruits was the highest in “Akihime,” followed by “Benihoppe,” “Tochiotome,” and “Sachinoka.” The number of fruits of “Akihime” was significantly higher than those of “Tochiotome” and “Sachinoka” (Fig. 3B). “Akihime” had the highest number of flowers, followed by “Benihoppe,” “Tochiotome,” and “Sachinoka.” The total numbers of flowers of “Akihime” and “Benihoppe” were significantly higher than those of “Tochiotome” and “Sachinoka” (Fig. 3C). “Benihoppe” had the highest number of inflorescences, followed by “Akihime,” “Tochiotome,” and “Sachinoka.” The total numbers of inflorescences of “Benihoppe” and “Akihime” were significantly higher than those of “Tochiotome” and “Sachinoka” (Fig. 3D).

**Fig. 3** (A) Fruit yields, (B) number of fruits, (C) number of flowers, and (D) number of inflorescences of four June-bearing strawberry cultivars grown in a plant factory with artificial light. *Data are the mean (± standard deviation (SD)) of three independent measurements taken from May 10 to October 31, 2013. Different letters, a, ab, and b, indicate significant differences at P < 0.05 using the Tukey-Kramer test.

### Table 1 Effects of artificial light intensity on the yields of “Benihoppe” cultivar.

| Light intensity | Total fruit yield (g/plant) | Number of fruits (plant) | Average fruit weight (g) |
|-----------------|----------------------------|--------------------------|--------------------------|
| Weak            | 318.8                      | 32.3                     | 9.9                      |
| Strong          | 443.5                      | 39.3                     | 11.3                     |

* Asterisk * and n.s. indicate significant and a non-significant difference, respectively, at P < 0.05 by t-test.

16:00. The average photosynthetic rates in strong and weak light treatments between 10:00 and 17:00 were 7.31 μmol m⁻² s⁻¹ and 5.41 μmol m⁻² s⁻¹, with variation coefficients of 0.18 and 0.11, respectively.

**DISCUSSION**

In Experiment 1, we investigated if transferring June-bearing strawberry plants into a PFAL after forced greenhouse cultivation in winter and spring was good for continuous production in summer and autumn. The lowest monthly yields were recorded from April to July in the greenhouse among the three treatments (Fig. 2). Flower buds differentiation presumably remained until May in the greenhouse, because strawberries usually take 2 months for a fruit to mature. During this period, the strawberry plants in the greenhouse were exposed to over 14-hour day lengths in mid-June (National Astronomical Observatory HP), and the greenhouse temperature exceeded 30°C since
May. Flower bud differentiation did not occur due to long days and high temperatures, leading to no harvested fruits in August. In addition, the yield in April should not change significantly in the three treatments, but the yield in the greenhouse treatment was lower than that in the other two treatments. This was because the experiment was started on April 11, and the yield in the greenhouse treatment was high until April 11.

On the other hand, in LS and MM treatments, flower bud differentiation, flowering, and fruit development continued after June, and strawberries were harvested in August. Comparing LS and MM treatments, there was no difference in yield between April and May, but there was a difference in yield after June. It is presumed that in April and May, in LS and MM treatments, flower bud differentiation had already occurred before transferring to the PFAL. The yield after June was higher when the plant was grown in an environment of LS treatment where reproductive growth was induced than in an environment of MM treatment where vegetative growth was promoted.

In Experiment 1, it was found that cultivation could be continued until the end of August by transferring the plants to a PFAL. In Experiment 2, to determine if the treatment was applicable for year-round production of June-bearing strawberries, we extended strawberry production for 2 months in LS conditions and 3 months in MM conditions. Furthermore, we attempted to identify cultivars suitable for PFALs by comparing their yields.

In previous studies, “Benihoppe” and “Akihime” had higher fruit yields than those of “Tochiotome” and “Sachinoka” (Shizuoka Prefectural Research Institute of Agriculture and Forestry, 2005; Takahashi et al., 2010; Yamazaki et al., 2010; Kato et al., 2015). In this study, “Benihoppe” also had the highest fruit yield, followed by “Akihime,” “Tochiotome,” and “Sachinoka.” This means that the ecological characteristics of cultivars related to yield are not different, even under the stable conditions in a PFAL, or in a greenhouse where temperature and humidity fluctuates. “Benihoppe” and “Akihime” had significantly higher numbers of inflorescences, flowers, harvested fruits, and fruit yields than those of the other two cultivars (Fig. 3). In this study, we did not investigate the dry matter and leaf area; Mochizuki et al. (2013) discussed that the tall “Benihoppe” cultivar demonstrates more vigorous growth than that of the others because of its morphology that allows more light to penetrate the whole plant. As “Akihime” and “Benihoppe” are upright and can efficiently use light under weak light intensity, they are suitable for cultivation in PFALs. “Benihoppe,” characterized by vigorous and sustained differentiation of inflorescences and flowers, had higher fruit yields. In future studies, it is necessary to investigate the yield, fruit quality, and production cost of the recommended cultivars in detail, with an increased number of samples to assess their economic viabilities.

In Experiment 3, to increase the yield of “Benihoppe,” which had the highest yield in Experiment 2, weak light, which was used in Experiment 2, and strong light treatments were set up. The total fruit yield and average fruit weight were significantly higher in the strong light treatment than in the weak light treatment (Table 1).

In addition, when the PPFD was 300–500 μmol m$^{-2}$ s$^{-1}$, stronger light led to higher yield of “Benihoppe.” This was considered to be due to the increase in leaf area or net assimilation rate related to the photosynthetic rate of leaves. Therefore, the comparison of diurnal changes in leaf photosynthetic rates of “Benihoppe” under weak and strong light conditions in the PFAL in July was made (Fig. 4). Leaf photosynthetic rate slightly decreased at 16:00 in the strong light treatment, but it was almost constant in both treatments, regardless of the duration of irradiation. The photosynthetic rates of “Tochiotome” grown in the greenhouse increased and climaxed in the morning and decreased over time in the afternoon (Wada et al., 2010). This observation was attributed to a decrease in stomatal conductance, as the water potential of the leaves decreased in the afternoon (Inaba, 2007). However, the light saturation point of “Benihoppe” is 1,000 μmol m$^{-2}$ s$^{-1}$ or more in an environment with a CO$_2$ concentration of around 400 ppm (Hidaka et al., 2017), the light intensity of the PFAL was about 30 to 50% of the light saturation point of “Benihoppe.” Furthermore, it was cultivated under conditions where temperature and humidity were constant, with regular nutrient supply and low water stress. The photosynthetic rate of “Benihoppe” cultivated in weak and strong light treatments became constant as a result of photosynthesis of leaves under the condition of no light saturation and low water stress.

Comparing the weak and the strong light treatments (Fig. 4), the photosynthetic rate of the leaves increased significantly in strong light treatment throughout the day, and the coefficient of variation was greater than that in weak light treatment. This suggests that the photosynthetic rate
of leaves of strawberries cultivated in an environment with high light intensity increases the daily fluctuation, and this should be confirmed by experiments with a higher light intensity.

In addition, fruit yield was also higher in strong light treatment (Table 1). Higher artificial light intensity in the plant factory directly led to higher leaf photosynthesis and fruit yield, as photosynthesis was promoted during the fruit coloring period and a large amount of photosynthetic products were translocated to the fruits. Nishizawa and Hori (1988) reported that about 50% of the photosynthetic products of the leaves were translocated, of which 86% were translocated to the fruits in the fruit coloring period. In the future, it is presumed that more efficient light conditions can be identified by studying the translocation dynamics of photosynthetic products in each growth stage. In addition, changes in photosynthesis-temperature curve are expected to examine in detail the photosynthesis of "Benihoppe" and the effects of temperature and other growing environments.

June-bearing strawberry plants demonstrated their extended production potentials in the PFAL under the favorable flower budding conditions. It became clear that “Benihoppe” had high yield even in the PFAL, with the temperature of 20/10°C (light/dark period), 8 hours of day length, and PPFD of 500 μmol m⁻² s⁻¹, and the fruit production continued until October. In this study, we focused on whether the strawberry plants used for forcing cultivation could be stored and continuously cultivated in a PFAL. In the future, it will be necessary to analyze the quality, production cost, and growth of the entire plant in implementing this technique.

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