Effect of Different PPFDs and Photoperiods on Growth and Yield of Everbearing Strawberry ‘Elan’ in Plant Factory with White LED Lighting

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In this study, we investigated the effect of continuous lighting for efficient plant growth during the reproductive period of everbearing strawberry (Fragaria×ananassa Duch. ‘Elan’) in a plant factory with white LED lighting. The plants were grown under different photoperiods (16 and 24 hours per day) and PPFDs (200, 300, 400, and 500 µmol m⁻² s⁻¹). The fruit yield was significantly lower at 200 µmol m⁻² s⁻¹ with either photoperiods and was significantly higher under 24 hours than 16 hours photoperiod. The rate of yield increase by intensifying PPFD was higher under 24 hours than under 16 hours photoperiod. The soluble solids and ascorbic acid content was significantly lower at 200 and 300 µmol m⁻² s⁻¹ with 16 hours photoperiod and 200 µmol m⁻² s⁻¹ with 24 hours photoperiod, but not different between the photoperiods. The anthocyanin content was significantly higher under 24 hours than under 16 hours photoperiod. The crop’s productivity (g mol⁻¹) was higher under 24 hours than under 16 hours photoperiod and 300 µmol m⁻² s⁻¹ was higher than that of different PPFDs. These results indicate that continuous lighting at 300 µmol m⁻² s⁻¹ is more efficient for cultivating everbearing strawberry.

Keywords: anthocyanin, ascorbic acid, continuous lighting, DLI, productivity

INTRODUCTION

In recent years, plant factory with artificial light (PFAL) has become popular in Japan (Goto, 2012). The important characteristics of the plants suitable for PFAL are as follows: (1) the plant’s height must be relatively low, (2) the time from sowing to harvesting or coming to maturity should be short, and (3) the plant should be able to grow even at low-light intensity. Therefore, previous studies related to vegetable production in PFAL often used leafy vegetables and seedlings (Kozai, 2018). However, strawberry plant has lower light requirement and lower plant height than tomato and cucumber, so it can be cultivated in a multi-layer system in PFAL. In addition to this, the unit price of strawberry fruits is higher than that of leafy vegetables. Hence, it is considered to be suitable for strawberry production in PFAL (Yoshida et al., 2013).

In recent PFALs, light emitting diode (LED) is usually used as the light source. LEDs have several advantages over fluorescent lamps. For example, the quality of light can be controlled by adjusting the arrangement of the emitting element, the lifetime is long, the amount of light is stable for a long time, light emits no heat, and so on. Concerning the quality of light, many experiments using LEDs have been conducted in environmentally controlled closed-type chambers. Among them, a mixture of red and blue has been reported to be efficient for plant cultivation (Nhut et al., 2003; Shin et al., 2008). Moreover, Lin et al. (2013) reported that the addition of white LEDs to red-blue mixed LEDs promoted lettuce growth. Regarding studies on strawberries, though there have been studies conducted on the effect of light quality, that is, red, blue, or red-blue mixed on strawberry plants in a closed plant factory (Choi et al., 2015; Yoshida et al., 2016), there is limited information on the suitability of white LEDs in strawberry production.

Considering strawberry production in PFAL, everbearing varieties may be more suitable than June-bearing varieties (Yoshida et al., 2013). June-bearing varieties require low temperature with short-day condition for flower bud differentiation, whereas everbearing varieties require long-day condition for the same. Thus, the growth and continuous flowering of everbearing varieties can be simultaneously promoted under long-day condition. It is considered to lead to shortening of the seedling period. For this reason, several studies on photoperiod using everbearing varieties have been carried out and continuous lighting with blue light was efficient for running cost and initial cost (Yoshida et al., 2012; Yoshida et al., 2016), however, there is limited information about how much light (daily light integral; DLI), that is, the product of photosynthetic photon flux density (PPFD) by day’s length, the strawberry actually needs under continuous lighting. Therefore, in this experiment, we set various DLIs by combining different photoperiods and PPFDs, and evaluated the continuous light with white LED.
MATERIALS AND METHODS

Cultivation method
Everbearing strawberry (Fragaria×ananassa Duch., ‘Elan’) was used in this study. The seeds were sown in a cell tray (105 cell per tray, 545×280 mm) filled with vermiculite and the seedlings were raised for 45 days under controlled environmental condition. The room temperature was maintained at 25°C and the PPFD was 300 μmol m⁻² s⁻¹ at the plant canopy for 16 hours photoperiod by using white LED (NO343B; ODELIC Co., Ltd., Tokyo, Japan) (Fig. 1). The plants were irrigated with a nutrient solution (Tank Mix F&B solution; EC, 0.8 dS m⁻¹; OAT Agrio Co., Ltd., Tokyo, Japan) once in two days at 10:00 from 6 days after sowing. Then 6 seedlings were transplanted (10 plants per m²) to a container (1,200 mm long×200 mm wide×100 mm high) in a row with non-woven fabric at the bottom under nutrient film technique (Fig. 2). The room was maintained at 23°C, then treatments were started. The treatments consisted of different photoperiods and PPFDs with the same white LED used for seedlings stage. The photoperiods were 16 hours and 24 hours, and PPFDs at the plant canopy were 200, 300, 400 and 500 μmol m⁻² s⁻¹ using different LEDs and adjusting the height from the bottom and the CO₂ concentration of 400 μmol mol⁻¹. The plants were irrigated with a nutrient solution (Tank Mix F&B solution; EC, 1.2 dS m⁻¹; OAT Agrio Co., Ltd., Tokyo, Japan) twice a day at 10:00 and 18:00. One container with 6 plants was used for each treatment, and the treatment was replicated twice. Data were collected from 12 plants (6 plants from each replicate). The cultivation period was 125 days from the day of transplanting and harvest period was 60 days. The pollination was conducted with a brush.

Measurement of crop productivity, fruit yield and quality
Fruits were harvested at the red-ripen stage and over 5 g were defined as marketable. Crop productivity was calculated from cumulative yield of individual plant over cumulative PPFD within the harvest period. 20 plants were used for the soluble solids content (TSS) and the total acidity (TA). The TSS was measured using a digital refractometer (PAL-1; Atago Co., Ltd., Tokyo, Japan). The TA was measured by titration with 0.05 N NaOH and expressed as the percentage of citric acid. Three fruits were used for each measurement with three repetitions. Fruit samples were stored at −25°C until measurement. Approximately 5.0 g of fruit was ground with 5 mL ethanol acidified with 1% (v/v) HCl, then the suspension was shaken at 120 rpm for 24 hours and centrifuged at 2,400×g for 20 minutes. The supernatant was used for the measurement of total anthocyanin content using a spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at a wavelength of 510 nm. The results were expressed as milligram of cyaniding 3-glucoside equivalents per 100 g of fresh weight. Approximately 3.0 g of fruit was ground with 1% metaphosphoric acid solution. The extract was used to determine the total ascorbic acid content using RQ flex (Merck Co., Ltd., Darmstadt, Germany).

Statistical analysis
Significant differences in plant growth, yield, and fruit quality were tested by analysis of variance, and the significant differences among means were tested by Tukey Kramer test at $P < 0.05$.

RESULTS
The number of inflorescences increased under 24 hours photoperiod than under 16 hours, and it was significantly lower at 200 μmol m⁻² s⁻¹ compared to that under...
EFFECT OF DIFFERENT PPFDs

Table 1  Number of inflorescences, number of branch crowns, yield, marketable yield, number of fruits, and fruit weight of everbearing strawberry ‘Elan’ grown at different PPFD with 16 hours and 24 hours photoperiod.

| Photoperiod (h day⁻¹) | PPFD (µmol m⁻² s⁻¹) | Number of inflorescences | Number of branch crowns | Yield (g/plant) | Marketable yield (g/plant) | Number of fruits | Fruit weight (g/plant) |
|-----------------------|----------------------|--------------------------|-------------------------|----------------|--------------------------|-----------------|------------------------|
| 16 h                  | 200                  | 5.7±0.4 b                 | 2.3±0.3 b               | 155±11 b       | 127±12 b                  | 93.3±4.0 a      | 93.3±4.0 a             |
|                       | 300                  | 9.7±0.8 a                 | 4.3±0.6 ab              | 237±15 a       | 210±16 a                  | 93.3±4.0 a      | 93.3±4.0 a             |
|                       | 400                  | 10.8±0.9 a                | 5.5±0.8 a               | 226±14 a       | 191±15 a                  | 93.3±4.0 a      | 93.3±4.0 a             |
|                       | 500                  | 9.7±0.5 a                 | 4.5±0.3 a               | 261±20 a       | 233±17 a                  | 93.3±4.0 a      | 93.3±4.0 a             |
| 24 h                  | 200                  | 9.8±0.6 b                 | 3.7±0.3 a               | 247±34 b       | 203±35 b                  | 93.3±4.0 a      | 93.3±4.0 a             |
|                       | 300                  | 13.5±1.2 ab               | 4.7±0.5 a               | 410±42 ab      | 349±37 ab                 | 93.3±4.0 a      | 93.3±4.0 a             |
|                       | 400                  | 16.0±1.3 a                | 5.2±0.4 a               | 460±53 a       | 413±49 a                  | 93.3±4.0 a      | 93.3±4.0 a             |
|                       | 500                  | 13.3±1.2 ab               | 6.0±1.3 a               | 512±38 a       | 467±38 a                  | 93.3±4.0 a      | 93.3±4.0 a             |

Table 2  Total soluble solids (TSS), titratable acid (TA), sugar-acid ratio, ascorbic acid, and anthocyanin of everbearing strawberry ‘Elan’ at different PPFD with 16 hours and 24 hours photoperiod.

| Photoperiod (h day⁻¹) | PPFD (µmol m⁻² s⁻¹) | TSS (% Brix) | TA (%) | Sugar-acid ratio | Ascorbic acid (mg/100 g FW) | Anthocyanin (mg/100 g FW) |
|-----------------------|----------------------|--------------|--------|-----------------|----------------------------|--------------------------|
| 16 h                  | 200                  | 7.6 b        | 1.0 a  | 7.3 a           | 53.3 c                     | 31.1 a                   |
|                       | 300                  | 7.4 b        | 1.0 a  | 7.6 a           | 53.0 c                     | 48.3 a                   |
|                       | 400                  | 8.5 a        | 1.1 a  | 8.0 a           | 65.7 b                     | 37.7 a                   |
|                       | 500                  | 8.4 a        | 1.1 a  | 7.7 a           | 74.0 a                     | 35.6 a                   |
| 24 h                  | 200                  | 7.4 c        | 0.9 b  | 8.0 a           | 56.3 b                     | 62.5 a                   |
|                       | 300                  | 8.4 b        | 1.1 a  | 7.8 a           | 70.7 a                     | 61.2 a                   |
|                       | 400                  | 8.9 a        | 1.2 a  | 7.7 a           | 68.7 a                     | 62.7 a                   |
|                       | 500                  | 8.6 ab       | 1.1 a  | 8.0 a           | 71.0 a                     | 62.4 a                   |

The crop productivity decreased as increasing PPFD under both photoperiods. In comparison with photoperiods, the plants under 24 hours was significantly higher than that of plants under 16 hours at 400 and 500 µmol m⁻² s⁻¹ (Fig. 3) (P < 0.001; t-test). The crop productivity was higher in the plants with 300 µmol m⁻² s⁻¹ in the both photoperiods. In the plants under 16 hours photoperiod, the TSS was significantly lower at 200 and 300 µmol m⁻² s⁻¹ with 16 hours photoperiod and not different between 400 and 500 µmol m⁻² s⁻¹ (Table 2). The TSS was lower at 200...
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\[ \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \]

with 24 hours photoperiod and not different between 400 and 500 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \). TA was not significantly different among the PPFDs with 16 hours photoperiod but significantly lower at 200 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \) with 24 hours photoperiod. The ascorbic acid content was lower at 200 and 300 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \) with 16 hours photoperiod and at 200 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \) with 24 hours photoperiod. There was significant difference among the photoperiods and PPFDs. The anthocyanin content was not different among the PPFDs but was significantly different between the photoperiods (\( P < 0.001 \); Table 2).

DISCUSSION

Some studies have shown that longer photoperiods promote the flowering and higher temperatures inhibit the flowering of everbearing strawberries (Kumakura and Shishido, 1995; Nishiyama et al., 2003). Simstebey et al. (2007) and Hamano et al. (2015) reported that continuous lighting increased the number of inflorescences in strawberry plants. In this study, more inflorescences were also observed under 24 hours than under 16 hours photoperiod (Table 1) and there was no difference in flowering date under either photoperiod (data not shown). Moreover, there was a correlation between fruit yield and number of inflorescences (Fig. 4). These results suggested that increasing the number of inflorescences was connected to greater yield in the plants under 24 hours photoperiod.

In the present study, DLI was higher under 24 hours than 16 hours photoperiod at the same PPFD. Therefore, we analyzed the relationship between the yield and DLI at different photoperiods (Fig. 5). The yield had a positive correlation with DLI and the rate of yield increase with intensifying PPFD was higher under 24 hours than 16 hours photoperiod. Yoshida et al. (2013) reported that when DLI was the same, the assimilates were delivered to fruits more efficiently under 24 hours than 16 hours photoperiod. In the present study, the plant vigor by appearance was higher with 16 hours than with 24 hours (data not shown), suggesting that more assimilates were delivered to fruits under 24 hours than 16 hours photoperiod.

In the crop productivity, the plants under 24 hours was higher than 16 hours (Fig. 3). In the plants under 16 hours photoperiod, the crop productivity was significantly lower under 400 and 500 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \) than 200 and 300 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \). Kumakura and Shishido (1994) showed that the rate of assimilate distribution to fruit was lower at 25°C daily air temperature than that at 20°C and 15°C in strawberry plants, regardless of temperature difference between day and night. In our study, the air temperature was 23°C and luxuriant growth was observed under 400 and 500 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \) with 16 hours photoperiod. Moreover, Garcia and Kubota (2017) reported that the diurnal decline in photosynthesis could be caused by a negative feedback of photosynthesis associated with unbalanced demand and supply of photoassimilates when sink strength is low at the beginning of the harvest season and when source strength is very high towards the end of the season. Therefore, the reason for the decrease in the crop productivity under 400 and 500 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \) with 16 hours photoperiod could be caused by the lower rate of assimilate partitioning to fruit and unbalanced sink source ratio, which decreased the photosynthesis.

In the plants under 24 hours, the crop productivity decreased with increasing PPFD more than 300 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \) (Fig. 5). The symptom caused by photoinhibition under extended period of continuous lighting was reported for several plant species (Demers et al., 1998; Hata et al., 2012; Wolff and Langerud, 2006). In our study, the symptom (necrosis) was observed under 300, 400, and 500 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \) with 24 hours photoperiod and the degree of symptom was higher with increasing PPFD (Fig. 6). In fact, although the yield was higher under 400 and 500 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \) than 300 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \), the increase in yield under 400 and 500 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \) compared to that under 300 \( \frac{\text{mol m}^{-2} \text{s}^{-1}}{\text{h}} \) (12%, 25%, respectively) were less than expected based on the increase in the daily light integral (DLI, +33%, +67%, respectively). Therefore, decrease of
crop productivity under 400 and 500 μmol m$^{-2}$ s$^{-1}$ with 24 hours photoperiod may be due to photoinhibition.

The TSS was significantly lower under 200 and 300 μmol m$^{-2}$ s$^{-1}$ with 16 hours photoperiod and under 200 μmol m$^{-2}$ s$^{-1}$ with 24 hours photoperiod. Hidaka et al. (2013) reported that photosynthate production was higher under LED supplemental lighting, resulting in increased TSS. Therefore, the decreased TSS at lower PPFDs in the present study may be owing to the effect of low photosynthate production.

The anthocyanin content was significantly higher under 24 hours than 16 hours photoperiod with different PPFDs at 120 days after transplanting. (a) indicate the leaves with 500 μmol m$^{-2}$ s$^{-1}$ and (b) indicate the leaves with 300 μmol m$^{-2}$ s$^{-1}$.

**Fig. 6** The symptom caused by photoinhibition under 24 hours photoperiod with different PPFDs at 120 days after transplanting. (a) indicate the leaves with 500 μmol m$^{-2}$ s$^{-1}$ and (b) indicate the leaves with 300 μmol m$^{-2}$ s$^{-1}$.

**CONCLUSION**

The plants were grown under different photoperiods (16 and 24 hours per day) and PPFDs (200, 300, 400, and 500 μmol m$^{-2}$ s$^{-1}$) with white LED. The yield, TSS, ascorbic acid, anthocyanin and crop productivity of everbearing strawberry ‘Elan’ were significantly higher under 24 hours than 16 hours photoperiod. In the plants under 24 hours, the yield was not different among 300 to 500 μmol m$^{-2}$ s$^{-1}$ and the crop productivity was higher with 300 μmol m$^{-2}$ s$^{-1}$. Therefore, the continuous lighting of everbearing strawberry was efficient compared with 16 hours, but over 300 μmol m$^{-2}$ s$^{-1}$ was inadequate due to photoinhibition.

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