Influence of light quality on flowering characteristics, potential for year-round fruit production and fruit quality of blueberry in a plant factory

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Summary

Introduction — Blueberry harvest season is only from June to September in Japan. Developing new cultivation methods for year-round blueberry production is desired to reduce off-season blueberry imports at a high price. Consulting with previous studies about influences of environmental factors on plants, the current study was carried out to investigate flowering characteristics, plant morphology, and potential for year-round high quality blueberry production under different light quality in a plant factory. Materials and methods — Blueberry plants cvs. ‘Misty’ and ‘Sharpblue’ were grown in light emitting diodes (LED) chambers installed with LED of 100% blue (459 nm), 100% red (631 nm), a mixture of 1:1 red:blue light (a mixture of LED lights). Fluorescent light was used as control. Results and discussion — Cumulative flower number was maximum in ‘Misty’ under blue LED light and in ‘Sharpblue’ under a mixture of LED lights. In ‘Sharpblue’, red LED light encouraged vegetative growth, and plants under blue light were delayed in growth with cessation of shoot elongation. However, a mixture of LED lights and fluorescent ones showed desirable vegetative growth and high potential for continuous flowering. In terms of fruit quality, higher soluble solid content (SSC) and less titratable acidity (TA) were found in fruits under blue LED light and a mixture of LED lights. Bigger fruits were found in plants under a mixture of LED lights and control but comparatively less SSC was found under control. Conclusion — Light quality has been shown to have a strong influence on flowering characteristics, plant morphology, and year-round high quality blueberry production in a plant factory.

Keywords
blueberry, Vaccinium corymbosum, flower development, light quality, plant growth, controlled environment

Introduction
Blueberry belongs to family Ericaceae, genus Vaccinium and it has approximately 400 species (Vander Kloet, 1988). Among fruits, blueberries are considered to be rich in phenolic compounds and aware due to its high antioxidant activity scores, flavor, color and nutritional properties (Diaconeasa et al., 2015; Giacalone et al., 2015; Zafra-Stone et al., 2007).

The origin of blueberry is the United States of America (Leisner et al., 2017) and blueberry was introduced into Japan in 1951 (Tamada, 2006). There are many benefits of blueberry for human health (Giacalone et al., 2015) and therefore, Japanese consumers’ demand on blueberry has also increased year by year. In 1996, due to increased demand on blueberry, production area and local production increased but the amount of import also increased and it was over 50% of domestic production (Trade Statistics of Japan, 2015). Domestic production did not meet Japan market demand because the harvest season of blueberry fruit in Japan takes only for around 4 months (only from June to September) in the open-field production and Japan needs to import tons of fresh and frozen blueberry, especially during off-season, at a high price. Therefore, Japanese farmers tried to extend the blueberry harvest period using artificial heating systems in plastic houses, which enabled the blueberry harvest to start from late March (Higashide et al., 2006; Tokyo metropolitan wholesale market, 2018). However, year-round production of blueberry fruit, including the off-season (October to March), is still desired in Japan. Therefore, this current
study was conducted to develop new cultivation methods for year-round blueberry production in Japan. Plant factories have great potential for hyper-yield, superior product quality, off-season production, shorter production period and so on. Therefore, the current study was conducted in an environmental control room of a plant factory of Tokyo University of Agriculture and Technology (TUAT) using light emitting diodes (LED) equipped chambers. The TUAT plant factory has six environmental control rooms which simulate the four seasons of Japan, mainly focusing on blueberry year-round production (Ogiwara and Arie, 2010).

Previous studies discovered that light plays important roles in not only plant morphogenesis process, but also photosynthesis process (Long et al., 2015; Yamori, 2013; Yamori and Shikanai, 2016; Yamori et al., 2016; Zhu et al., 2010). Especially, plant factories mostly rely on artificial light for plant growth and development and fluorescent lamps have been used since long time ago. Nowadays, LED has become very popular in modern horticulture due to its abundant beneficial properties of energy saving, convenience of use, easy control of light spectrum – with narrow and specific wavebands – intensity and photoperiod, that all have direct influential effects on plant physiology, flowering characteristics and plant morphogenesis (Demotes-Mainard et al., 2016; Fan et al., 2013; Fialta and Carvalho, 2015; Lin et al., 2015; Singh et al., 2015). For example, red spectrum decreased flower numbers and delayed flowering in ‘Strawberry’ ( Taken-da et al., 2008); or phytochromes (phy) encouraged vegetative growth in Indian mustard and other five cultivars of basil ( Tarakanov et al., 2012; Endo et al., 2013). In contrast, some researchers mentioned the influence of blue light on reproductive phase: blue LED light promoted flowering in strawberry ( Yoshida et al., 2012); in petunia in closed-type environment (Fukuda et al., 2011); controlled flowering and bud outgrowth (Huché-Thélier et al., 2016). Moreover, previous findings pointed that blue light inhibited stem extension in Brassicaceae (Kigel and Cosgrove, 1991; Liscum et al., 1992), produced smaller leaves in salvia ‘vista red’ than those under red light spectrum and other combinations of lights (Runkle, 2017), smallest stem diameter and least net photosynthetic rate in tomato seedlings ( Yang et al., 2018). However, there is little information about a combination of blue and red light effects on flowering in blueberry under control environment.

Although information is limited about blueberry year-round production in plant factories from other parts of Japan or other places, some information was obtained from previous experiments of TUAT plant factory. Horuchi et al. (2013) and Ogiwara et al. (2012) reported that blueberry harvesting two times in one year is possible by accelerating plants’ life cycle in controlled rooms with artificial lighting systems in TUAT plant factory. Furthermore, Thanda et al. (2014) has also pointed that blueberry plants grown under artificial light in the TUAT plant factory could produce high quality fruits which are bigger, with higher SSC and lower TA compared to plants under natural sunlight. However, it is still desired to develop new cultivation methods for blueberry year-round production using different light quality.

The previous studies mentioned above suggested that artificial light and other environmental factors had influential effects on plant morphology, flowering phenomenon and fruit quality in plants. Also, the previous TUAT plant factory experiments pointed that fluorescent light and temperature control influenced on flowering and plant life cycle, enabling to harvest twice a year with high quality in some blueberry cultivars. However, the influence of a specific light spectrum on plant morphology, flowering phenomenon and its fruit quality in blueberry in the plant factory has not been well understood. Therefore, this current study was carried out based on the current need of Japan blueberry production and aim of this research was to investigate the flowering characteristics, plant morphology, fruit quality and potential for year-round production in response to different light quality by using LED and fluorescent light in TUAT plant factory.

Materials and methods

Plant material

Two cultivars of 3-year-old blueberry plants were used in the experiments: ‘Misty’ and ‘Sharblue’. Both are southern highbush blueberry (Vaccinium corymbosum) commercial hybrids presenting very low chill requirements.

Environmental conditions

In this study, two experiments were carried out from 2014 to 2016. Experiment 1 was performed from 17.6.2014 to 10.10.2014. In Experiment 2, the first part was carried out from May 12th 2016 to July 6th 2016 for 55 days and the second part was carried out from July 7th to December 19th for 165 days. The light intensity was set 380 ± 10 μmol m⁻² s⁻¹ for Experiment 1 and 300 ± 10 μmol m⁻² s⁻¹ for Experiment 2 at the top of plant canopies in each treatment section (MLJ-14PAR Type 2; Environmental Measurement Japan Co. Ltd., Fukuoka, Japan). The mixture of peat moss and Kanuma soil was used as a soil medium and automated drip irrigation and nutrient fertigation system was applied (Otsuka AgriTechno Co. Ltd., Tokyo, Japan) directly to plants root zone about 250 mL per pot twice a day. pH range was adjusted between 4.0 and 4.5 with the help of pH reducing solution containing phosphate (PₐO₅) (Otsuka Agri Techno Co. Ltd., Tokyo, Japan). Electric conductance (EC) was controlled from 0.8 to 1.0 dS m⁻¹. For better pollination, black bumblebees were performed.

Experiment 1

In Experiment 1, ‘Misty’ 3-year-old plants were moved from open field to an environmental controlled room and grown in LED chambers with different light quality: 100% Red spectrum (631 nm); 100% Blue spectrum (459 nm); and fluorescent light (FHF 32 EX-N-H, Panasonic) as the control. The experimental room was set at 28 °C in daytime and 18 °C at night, 40 to 80% relative humidity and about 480 ppm CO₂. Key research parameters were flowering time and number of flowers in plants in response to different light wavelengths. The flowering time were examined and number of flowers were counted at 45, 60, 75, 90 and 110 days after treatment (DAT). When the flower petals were completely opened and before the color of petals changed from white into brown color, the number of flowers was counted. The counted flowers were marked with marker pen to avoid repeated data collection.

Experiment 2

Experiment 2 was divided into two steps. In the first step, the 3-year-old ‘Sharblue’ plants were brought into plant factory and grown under blue LED light alone from 12th May 2016 until flower bud differentiation. The experimental room was set with 14 hours photoperiod, 25 °C in daytime and 14 °C at night, 40 to 80% relative humidity and about 480 ppm CO₂. When flower bud differentiation started at 55
DAT, the second step of Experiment 2 was continued under 10 hours photoperiod and 18–22 °C day/5–8 °C night provided room. The experimental pots with 5 replicates were placed in 3 chambers installed with LED lamps of 100% red LED light (631 nm), 100% blue LED light (459 nm), or a mixture of 50% red and 50% blue LED light (456 nm). Fluorescent lamps were applied as the control. Flowering time, flower numbers, plant morphology, potential for continuous flowering and fruit quality responses to different light quality were investigated. Flowering data were collected as in Experiment 1.

As part of fruit quality parameters, fruit weight, diameter, size and firmness, and soluble solid content (SSC) and titratable acidity (TA) of fresh fruit juice (in °Brix and in %, respectively) were analyzed. Fully ripe blueberry fruit, as judged by fully blue color at the pedicel end, were harvested randomly from each treatment and fruit weight (in g) and diameter (in mm) were measured. Fruit firmness was measured with a rheometer (RT-3005 D; Rheotech Co. Ltd., Tokyo, Japan). The fruits were placed perpendicularly on the table of the machine and the probe was applied to the fruits. The force to penetrate (with 2 mm Φ) the fruit was measured as fruit firmness in kilogram force (kgf). The juice was analyzed by titrating with 0.01 N NaOH. The TA was expressed in % as g citric acid 100 mL−1 juice. For SSC analysis, five randomly harvested fully ripe fruits from each plant were ground and 1 g fruit pulp was diluted and homogenized with 3 mL distilled water. The diluted fruit juice was centrifuged at 13,000 rpm for 10 min and SSC was measured using a digital refractometer (model PR101; Atago Co. Ltd., Tokyo, Japan) standardized with distilled water. The Brix value was multiplied by 4 to obtain the obtained value. Three replications were conducted for SSC, TA and SSC:TA ratio.

Plant morphology and continuous flowering were also examined at 165 DAT by checking new shoots, leaves, flowers, immature and mature fruits in ‘Sharppblue’ plants under different light wavelengths.

Statistical analysis

Plant morphological characteristics and fruit quality data in response to different light wavelengths were statistically analyzed by the Tukey Kramer’s test at P = 0.05 level after one-way ANOVA in Excel.

Results and discussion

Flowering time and number of flowers

The flowering delayed most in plants grown under red LED light, starting from 60 DAT (Figure 1). In contrast, the flowering time was earliest in plants under blue LED light, showing the first flowering date started at 45 DAT and it was followed by that of the plants under control. Flowering period in three treatments was for 110 days. Similarly in Experiment 2, flowering in ‘Sharppblue’ delayed most under red LED light but plants flowered the earliest in plants under a mixture of LED lights and it was followed by that of the plants under control and blue LED light (Figure 2). Therefore, it can be assumed that red light delayed flowering in both ‘Misty’ and ‘Sharppblue’. In addition, blue LED light encouraged early flowering most in ‘Misty’ (Experiment 1), and flowering time was promoted more when blue light combined with red light.

![Figure 1.](image-url)
Such synergic effect influenced flowering time, resulting in earliest among treatments in 'Sharpblue' (Experiment 2).

The total flower number of 'Misty' plants at 110 DAT was highest under red light (almost 300 flowers), followed by the plants under red LED light (102 flowers) and the control (73 flowers) (Figure 1). The total flower number at 91 DAT was highest in 'Sharpblue' under a mixture of LED lights (382 flowers), followed by the plants under red LED light (276 flowers), blue LED light (259 flowers), and the control (184 flowers) (Figure 2).

Previous studies have reported that floral induction and flowering were late under red LED light across a range of species in both long-day (LD) and short-day (SD) plants because phyB inhibited in floral induction in both LD and SD plants (Childs et al., 1997; Goto et al., 1991; Lin, 2000; Mockler et al., 1999; Takeda et al., 2008; Tarakanov et al., 2012; Weller and Reid, 1993). The current study agrees with these findings and shows delayed flowering time in both 'Misty' and 'Sharpblue', with a comparatively lower number of flowers under red LED light (Figures 1 and 2). A reason could be that the phyB inhibited flowering in both 'Misty' and 'Sharpblue' under red light.

In contrast, flowering time was earlier and total flowers was highest under blue LED light in 'Misty' (Experiment 1). It has been reported that blue light promoted flowering in ever-bearing strawberry compared to that of the plants treated with red LED light (Yoshida et al., 2012); in Arabidopsis thaliana, indicating that blue light inhibited phyB action and promoted flowering in Arabidopsis (Eskins, 1992; Guo et al., 1998; Mockler et al., 1999). It can also be assumed that the cryptochrome (cry) promoted flowering and encouraged early flowering in 'Misty' blueberry, like in strawberry and Arabidopsis thaliana from previous findings.

It is still unclear whether flowering was regulated by cry1 or cry2 in the present study, until further investigations are performed at molecular level for photoreceptor mechanisms under different light wavelengths.

Blue light alone encouraged flowering and it was found that both flowering time and number of flowers in 'Sharpblue' were promoted more when red light combined with blue light and the greatest cumulative flower number was observed under a mixture of LED lights. It might be assumed that a mixture of LED lights provided positive synergic promotion effects on floral induction and flowering compared to irradiation with red LED light or blue LED light in this current study. The current study is in agreement with Stutte et al. (2009) and Yorio et al. (2001), who reported that a mixture of blue and red lights had more powerful effects on plant growth, biomass and development due to higher absorption of chlorophyll a and b.

Plant growth and potential for continuous flowering in response to different light qualities

Plants under red LED light were observed with bigger leaves, greater number of leaves and overgrowth in vegetative parts but few fruits and flower (Figures 3 and 4). Similarly, Experiment 2 revealed that plant canopy was bigger under red LED light and it may be due to the fact that axillary buds differentiated into vegetative buds and phy encouraged vegetative phase. Tarakanov et al. (2012) and Endo et al. (2013) also provided similar proof that vegetative growth was promoted by red spectrum in Indian mustard and other five cultivars of basil, and the reason was that the phy encouraged vegetative phase. Yoshida et al. (2016) also reported that number of leaves under red light was greater than those under blue light and red to blue light. Yanagi et al. (1996) also report-
**Figure 3.** Plant morphological characteristics of blueberry ‘Sharpblue’ in response to different light treatments: A. Fluorescent light (control); B. Mixed LED lights (50% red + 50% blue); C. Blue LED light; D. Red LED light. Yellow circles represent immature and mature fruits; blue circles represent new shoots and young leaves; red circles represent flowers and immature fruit.

**Figure 4.** Blueberry ‘Sharpblue’ plant shape at 165 days after treatment (DAT) under different light treatments: A. Fluorescent light (control); B. Mixed LED lights (50% red + 50% blue); C. Blue LED light; D. Red LED light. Scale bar = 30 cm.
ed that lettuce plants grown under red LED alone had more leaves and longer stems than plants grown under blue LED. In contrast, plant vigor was very weak under blue LED light, with very small leaves and new axillary buds not developed and undifferentiated. Past findings pointed that that blue light inhibited stem extension in Brassicaceae (Kigel and Cosgrove, 1991), and in red leaf lettuce (Johkan et al., 2010). Runkle (2017) informed that plants under 100% blue light produced smaller leaves in salvia ‘vista red’ than those under red light spectrum and other combinations of lights. Yang et al. (2018) also provided the information about the effects of blue light on morphogenesis and photosynthetic pigment content in tomato seedlings, showing smallest stem diameter and least net-photosynthetic rate, less accumulation efficiency of chlorophyll a and b than those under red LED light and a mixture of red and blue LED.

Plants under a mixture of LED lights and control were observed with new shoots, healthy leaves, mature fruits, immature fruits, and flowers, which are positive results for continuous flowering year-round. Under mixed LED lights, the apical buds differentiated into flower buds because blue light enhances reproductive phase and desirable plant shapes were also observed because red LED light encouraged vegetative growth.

Finally, at 165 DAT, the potential for continuous flowering was examined and it was found that plants had high potential for continuous flowering under control and a mixture of LED lights (Figure 3) because plants had new shoots, young leaves, immature fruit, mature fruit and flowers. However, potential for continuous flowering was very low in plants under blue light which had no new shoot and flower; and under red LED light which had overgrowth and flowering almost stopped (Figures 2 and 3). Therefore, it can be assumed from this experiment that continuous irradiation with monochromatic light did not favor continuous flowering in ‘Sharpleaf’ plants. However, flowers, new shoots, young leaves, immature and mature fruits were observed in plants under control (fluorescent light) and a mixture of LED lights, showing positive results for continuous flowering.

Fruit quality responses to different light quality

Fruit size under a mixture of LED lights and control was significantly bigger than that of the plants under red LED light and blue LED light (Table 1). It might be due to the strong influence of a combination of LED light wavelengths on growth of crops than monochromatic wavelength, agreeing with past findings (Duong et al., 2003; Stutte et al., 2009; Yorio et al., 2010). In addition, the total chlorophyll amount was greater under both red LED alone and a combination of red and blue LED light whereas blue light had comparatively less efficiency to accumulate chlorophylls. Thus, fruit yield was lowest. This study was conducted by Choi et al. (2015). Yang et al. (2018) also provided the message that fruit size was smallest and yield was lowest under blue LED among treatments and control. This current study agreed with Choi et al. (2015) and Yang et al. (2018), although the total yield was not investigated.

Fruit firmness value was higher in plants under blue LED light and control compared to that of the plants under a mixture of LEDs and red LED light, respectively. Fruits in plants under blue LED light and a mixture of LED lights had significantly higher SSC than that of the fruits under control and red LED light: highest SSC in fruits under a mixture of LED lights and lowest under red LED light. Fruits under red LED light had the highest TA and it was followed by that of the fruits under a mixture of LED lights, blue LED light and control, respectively. The sugar acid ratio was the highest in the control group, followed by a mixture of LED lights group, the blue light group, and red light group, respectively.

Previous findings indicated that blue LED light increased the total sugar content in strawberry (Xu et al., 2014) and sugar and starch in in vitro growth of grape “Teleki 5BB” (Heo et al., 2006), their results were in agreement with our current study, indicating that blue light increased SSC in blueberry. However; the information about the influence of red LED light and a mixture of LED lights on acidity in fruits was limited. In this experiment, a mixture of LED lights increased SSC although red LED light decreased SSC most. According to Beaudry (1992), high-quality blueberry fruit should have SSC > 10 °Brix and TA between 0.3 and 1.3%. In this study, SSC was higher than 12 °Brix and TA was less than 1.3%, meaning that fruit tasted sweet and fruit quality was high under all treatments, including the control.

Table 1. Fruit characteristics of ‘Sharpleaf’ blueberry in response to different light treatments in TUAT closed-type plant factory. Data are mean values (n = 3). FL: Fluorescent light; LED: Light emitting diodes; SSC: Soluble solid content; TA: Titratable acidity.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Weight (g)</th>
<th>Size (mm)</th>
<th>Fruit firmness (kgf)</th>
<th>SSC (* Brix)</th>
<th>TA (%)</th>
<th>Sugar acid ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (FL)</td>
<td>2.2 a b</td>
<td>11.0 a</td>
<td>0.1670 a</td>
<td>12.7 b</td>
<td>0.30 b</td>
<td>42.3</td>
</tr>
<tr>
<td>Blue LED</td>
<td>1.6 b</td>
<td>9.7 b</td>
<td>0.1686 a</td>
<td>14.1 a</td>
<td>0.56 ab</td>
<td>25.2</td>
</tr>
<tr>
<td>Mixed LED light</td>
<td>2.1 a</td>
<td>11.2 a</td>
<td>0.1593 b</td>
<td>14.2 a</td>
<td>0.44 ab</td>
<td>32.3</td>
</tr>
<tr>
<td>Red LED</td>
<td>1.8 b</td>
<td>10.5 a</td>
<td>0.1537 b</td>
<td>12.8 b</td>
<td>0.65 a</td>
<td>19.7</td>
</tr>
</tbody>
</table>

*Values within the same column followed by different letters are significantly different at P=0.05 level using Tukey Kramer’s test after one-way ANOVA in Excel.
Experiments 1 and 2 revealed that it is possible to accelerate plant life cycle in ‘Misty’ and ‘Sharpleaf’ blueberries under different light quality in an environmental controlled room. Although blue LED light encouraged reproductive phase in both ‘Misty’ and ‘Sharpleaf’, it did not encourage plant growth after about seven months constant blue light irradiation. In contrast, red LED light delayed flowering in both cultivars and reduced flower number but overgrowth in ‘Misty’. However, it is assumed that adding blue light to red light could be more effective for early flowering, great flower number, big fruit with high SSC and low TA, desired plant growth and potential for continuous flowering.

Finally, this study confirmed that it is possible to produce high quality blueberry year-round under a mixture of LED lights in facilities like TUAT plant factory. Further studies are recommended to investigate molecular level photoreceptors and plant interactions under different light wavelengths.

References


