Shortening of the Juvenile Phase of the Southern Highbush Blueberry (Vaccinium corymbosum L. interspecific hybrid) Grown in Controlled Rooms under Artificial Light

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We attempted to shorten the juvenile phase of southern highbush blueberries by using controlled rooms under artificial light. Seeds were extracted from fresh fruits and sowed in the Woody Plant Medium (WPM) immediately after harvest. When these seeds were irradiated with red LED, their germination rate was over 80% by the 35 days after sowing. The seedlings in the controlled room continued to grow without entering the dormant phase. Especially, maximum growth was observed under the long-day condition of 12-h light. Even under the short-day condition of 8-h light, increased growth was observed when grown at a high light intensity (400 μmol·m⁻²·s⁻¹). For the seedlings that were moved from the long-day controlled room to the short-day controlled room after 10 months, the ‘Misty’ seedlings flowered within 327 days after sowing while the ‘Sharpblue’ seedlings flowered within 357 days after sowing. Furthermore, when cultivated under the short-day condition at 400 μmol·m⁻²·s⁻¹ for the entire length of the experiment, the flowering of ‘Misty’ seedlings was observed within 300 days after sowing. The seedlings maintained under the short day condition of high light intensity flowered earlier than seedlings moved from the long-day controlled room to the short-day controlled room. Thus, southern highbush blueberries were successfully induced to flower in less than a year from seed planting, using a combination of techniques to promote germination and control the growth environment conditions in a controlled room.

Key Words: controlled environment, initial growth, LED, seed germination.

Introduction

Fruit trees, which are perennial woody crops, have a long juvenile phase that initiates at the time of seed germination and continues until they first set fruits. Shortening the juvenile phase is highly desirable for efficient breeding. Commonly used techniques for shortening the juvenile phase include a technique to promote seed germination (Castro et al., 2012; Nin et al., 2017) and techniques to accelerate the growth of seedlings after germination (Nin et al., 2017). Early flowering was also achieved by grafting new shoots obtained from young seedlings (Mitani et al., 2008). For example, Kotoda et al. (2010) reported the juvenile phase in apple trees generally lasted from four to eight years or more, and other techniques have been developed to achieve flowering in two years by promoting growth and adapting grafting techniques (Kato et al., 2004). Transgenic apple plants were reported to have exhibited flowering within 1.5–2 months when the FLOWERING LOCUS T gene of Arabidopsis thaliana was introduced into them (Yamagishi et al., 2011). However, presently the use of transgenic technology is restricted to a limited number of species and/or cultivars of fruit trees, and even if such attempts were made, the procedures would be time-consuming and the transformation efficiency would be very low (Bulley et al., 2007).

In recent years, it has become possible to precisely control the environment in plant factories (Oshima et al., 2015). Techniques for promoting plant growth and controlling flowering time have been developed (Ogiwara et al., 2012; Oshima et al., 2015; Takatsuzi
and Mor, 2011). If environmental control can regulate seed germination, growth of germinated seedlings and flowering time, it may be possible to identify and maintain the precise environmental conditions under which the juvenile phase of fruit trees is drastically shortened. In the search for such environmental conditions, we studied the literature to find the conditions that were reported to promote seed germination, accelerate plant growth or control flowering time. First, there are a number of reports on the improved germination rate and early seed germination in *vaccinium* species. Exposure of seeds to low temperature before sowing improved the germination rate (Nin et al., 2017; Shigyou et al., 2014), and seeds that were extracted from fresh fruits and sowed immediately were shown to germinate successfully (Giba et al., 1995; Nin et al., 2017). Seeds irradiated with light had their germination accelerated (Giba et al., 1995; Nin et al., 2017). *In vitro* culture was effective for promoting germination and seed growth (Castro et al., 2012; Miyashita et al., 2009; Nin et al., 2017). Seed germination was shown to be enhanced by treatment with gibberellic acid and 6N-benzyladenine (Dweikat and Lyrene, 1989). Priming seeds with KOH solutions improved seed germination (Gao et al., 1998).

Also, there is a number of reports in which the growth of shoots was promoted and the number of leaves increased for several fruit trees. Long-day treatment increased the growth of shoots in the satsuma mandarin (*Citrus unshiu* Marcow) (Inoue, 1989), grape (*Vitis vinifera* × *V. labruscana*) (Kubota et al., 2001), and southern highbush blueberry (Spann et al., 2003). In grapes (Kubota et al., 2001) and the Japanese persimmon (*Diospyros kaki* Thumb.) (Kurahashi et al., 2002), the growth of shoots was enhanced with supplementary lighting. The Japanese pear (*Pyrus pyrifolia*) (Ito et al., 2014) and southern highbush blueberry (Kadowaki et al., 2015) were reported to have had shoot growth promoted when cultivated with red LED illumination, and under this condition the number of leaves also increased. Selection of culture media also affected growth (Nin et al., 2017; Ochmian et al., 2010). Northern highbush blueberries (*V. corymbosum* L.) grown in peat exhibited vigorous growth (Ochmian et al., 2010). The germinated seedlings of bilberries (*V. myrtillus* L.) showed vigorous growth when transplanted to a substrate containing commercial peat based acid substrate, perlite and sand (Nin et al., 2017). Cultivation in a heated greenhouse promoted vegetative growth of southern highbush blueberries (Ogden and Van Iersel, 2009). Treatment with gibberellin paste accelerated the development of the main shoots and trunks of stock in peaches (*Prunus persica* (L.) Batsch) (Hamana et al., 2013).

Furthermore, many other horticultural techniques are known to be effective in inducing flower bud differentiation and flowering. Grafting the seedling onto fruit-bearing trees (top grafting) (Suto and Kudo, 2007) or onto dwarfing rootstocks (Soejima et al., 1998) resulted in earlier flowering of apples. It was also shown to be possible to induce flowering in olives (Hackett and Hartmann, 1964) and satsuma mandarins (Inoue, 1989) by exposure to cool temperature. Heating cultivation also accelerated flowering in blueberries (Higashide et al., 2006; Horiochu et al., 2013a; Ogden and Van Iersel, 2009). Flower bud initiation appeared to be promoted under short-day conditions in satsuma mandarins (Inoue, 1989), northern highbush blueberries (Hall et al., 1963), rabbiteye blueberries (Darnell, 1991), and southern highbush blueberries (Horiochu et al., 2013b; Spann et al., 2003). Root pruning and uniconazole treatment accelerated flower bud differentiation in peach seedlings (Tsukahara et al., 2009). Light quality (wavelength) influenced floral initiation, for example, the shoot apices of Japanese pear trees formed flower buds when treated with far-red LED light (Ito et al., 2014). Blue LED light was also shown to promote flower bud initiation in southern highbush blueberries (Kadowaki et al., 2015). In 2011, an advanced plant factory was established at Tokyo University of Agriculture and Technology, Tokyo, Japan with the purpose of producing blueberry fruits all year round (Ogiwara and Arie, 2010). The factory consisted of six rooms (three glass-houses under natural sunlight and three controlled rooms under artificial light) that can replicate the four seasons of Japan. Subsequently, the techniques for controlling the timings of flower bud differentiation, flowering and the growth of new shoots of blueberries were developed at this factory based on previous studies (Horiochu et al., 2013b; Mayumi et al., 2012; Ogiwara et al., 2012; Thanda et al., 2014). Blueberries normally take at least 4 years from seed germination to flowering under natural conditions (Moore and Clark, 1997). It has been hypothesized that it may be possible to drastically shorten the juvenile phase by combining techniques for promoting seed germination and increasing seedling growth with reference to the above-mentioned methods, as well as the techniques used in the plant factory for controlling differentiation into flower bud and leaf buds, sprouting, the growth of new shoots, flowering, and fruiting developed by Ogiwara et al. (2012).

In this study, we attempted to shorten the juvenile phase of blueberries using controlled rooms with artificial lighting which enabled the use of combinations of different light qualities, growth media which accelerated seed germination, and methods for promoting the growth of germinated seedlings. This was achieved by controlling the environment or light intensity, and also techniques for promoting flower bud formation and flowering of seedlings.
Materials and Methods

1. The effect of medium and light quality on the in vitro germination rate of blueberry seeds

Seeds of open-pollinated fruits of southern highbush blueberries ‘Misty’ and ‘Sharpblue’ were used. The seeds of the two cultivars were sown on two different types of seed germination media, an agarose medium and the Woody Plant Medium (WPM), on August 15, 2013, and germinated under irradiation from two kinds of LEDs (red LED: $\lambda = 630$ nm and blue LED: $\lambda = 460$ nm; OKI Digital Imaging Co., Ltd, Tokyo, Japan). Eight plots were prepared to examine eight different treatments which were different combinations of cultivars (2 types), media (2 types), and light qualities (2 types).

Sowing was carried out as follows. The agarose medium was prepared with 1% agarose only, and the WPM medium contained WPM (Lloyd and McCown, 1980), 2% sucrose, and 1% agarose. After adjusting the pH to 5.7, each medium was sterilized using an autoclave at 121°C for 15 min, and dispensed in 90 mm plastic petri dishes at 20 mL each on a clean bench.

Seeds from fruits were taken out and sterilized with 70% ethanol for 10 min and then with sodium hypochlorite solution of 1% available chlorine for 5 min. Sterilized seeds were rinsed with sterile water to remove sodium hypochlorite solution on a clean bench. For each dish, 25 seeds were sown and each experiment was repeated three times.

The petri dishes on which the seeds were sown were placed in a controlled room of the Advanced Plant Factory in Tokyo University of Agriculture and Technology, Tokyo, Japan, and treated with two different kinds of light quality (red and blue LEDs), set at a light intensity of about 120 μmol·m$^{-2}$·s$^{-1}$ illuminated from the top of the plastic petri dishes. The environmental conditions of the experiment were 13–28°C, 60–80% RH, and 12-h light. To examine the transition of the seed germination rate, it was investigated every 7 days for 35 days after sowing. The seed germination rate was identified by an L8 orthogonal table and factor analysis was performed.

2. The effect of the environment on the growth and flowering of blueberry seedlings

Seeds from open-pollinated fruits of southern highbush blueberries ‘Misty’ and ‘Sharpblue’ were used. Taking into consideration the germination rate determined in the previous experiment, the best growing conditions (i.e. the WPM medium and irradiation with red LEDs) were applied for this experiment. The harvested seeds were sown on the WPM medium and irradiated with red LEDs for germination. Seeds of ‘Misty’ were sown on June 21, 2015, and seeds of ‘Sharpblue’ were sown on July 2, 2015, respectively. When the germinated and rooted seedlings of ‘Misty’ and ‘Sharpblue’ reached approx. 1 cm high, the seedlings were taken from the medium and washed with water to remove any residual medium attached to them. The seedlings were planted on a cell tray (size of each cell; 2 cm × 2 cm) containing well-fed peat moss medium on August 28 and September 10, respectively. The tray was placed in a foamed polystyrene box (35 cm × 25 cm × 15 cm) and the foamed polystyrene box was then covered with a plastic wrap. The seedlings were allowed to acclimatize by gradually removing the plastic wrap covering the foamed polystyrene box until it was fully removed after 4 weeks. The acclimated environmental conditions were at 13–28°C, 40–80% RH, 12-h light, and 300 μmol·m$^{-2}$·s$^{-1}$ light intensity. The light was provided by using fluorescent lamps (FHF32EX-N-H; Panasonic Co. Ltd., Osaka, Japan).

Thereafter, when the seedlings of ‘Misty’ and ‘Sharpblue’ reached approx. 5 cm high, they were transplanted from the trays to pots (10.5 cm diameter × 9.0 cm height) containing peat moss medium on November 6 and 10, respectively. These potted plants were used to assess the effect of four different environmental growth conditions on the shoot development and flowering of blueberry seedlings. The following growth conditions were tested: i) field condition; ii) controlled room with long day (9–22°C, 40–80% RH, 12-h light, and 300 μmol·m$^{-2}$·s$^{-1}$ light intensity); iii) glasshouse condition (10–30°C, no RH-regulation, natural photo-periods, and 300–1000 μmol·m$^{-2}$·s$^{-1}$ light intensity). For all treatments, plants were provided with nutrient solution prepared with Otsuka House No. 1, No. 2, and No. 5 that were diluted 2-fold (Otsuka AgriTechno Co. Ltd., Tokyo, Japan). The electric conductivity (EC) was about 1.0 mS·cm$^{-1}$, and pH was adjusted to 5.0 using a pH-reducing solution containing potassium phosphate (P$_2$O$_5$) (Otsuka AgriTechno Co. Ltd.). Irrigation was provided at 100 mL every 3 days.

Five seedlings were moved to each treatment plot on November 10, 2015 (which was the 142 days after sowing (DAS) for the seedlings of ‘Misty’, and the 131 DAS for seedlings of ‘Sharpblue’), and the shoot length, shoot number, and leaf number of these seedlings were measured on the 30, 60, 90, 120, and 150 days from November 10, 2015. The shoot number was the number of shoots generated per plant, and the leaf number was the average number of leaves per plant. Significant differences among the means were identified using the Tukey–Kramer test. In order to determine the flowering time, all seedlings were allowed to grow after the period of investigation (150 days during which the above described measurements were recorded) in the same field or artificial conditions, except for seedlings incubated in the controlled room with a long day, three seedlings each of ‘Misty’ and ‘Sharpblue’ were moved to the controlled room with a short day. The
number of days from sowing until flowering was counted from the day on which seeds were sown until the day on which the plants produced the first flower. The flowering rate was the ratio of the number of plants that flowered to the number of plants tested, which was measured on August 18, 2016 (on the 421 DAS for the ‘Misty’ seedlings, and on the 410 DAS for seedlings of ‘Sharpblue’). The flowers obtained in this experiment were pollinated artificially for subsequent experiments. The shoot length and leaf number per flowering seedling were measured on May 7, 2016 (on the 321 DAS for the ‘Misty’ seedlings, and on the 310 DAS for seedlings of ‘Sharpblue’) before the seedlings flowered. In this experiment, the number of seedlings investigated was as low as 3 due to the limited space in the controlled room.

3. The effect of light intensity on the growth and flowering of blueberry seedlings

Seeds from open-pollinated fruits of southern highbush blueberries ‘Misty’ were used. The seeds of ‘Misty’ were sown on April 30, 2014. The seedlings were cultivated for about 4 months after germination (until August 15) in the same acclimated environmental conditions as described in Experiment 2. From August 15, 2014 (which was the 107 DAS for the ‘Misty’ seedlings), the seedlings were moved and incubated in the environmental conditions described as “the controlled room with short day” in Experiment 2.

Three treatments with different light intensities were prepared at control intensities of 300 μmol·m⁻²·s⁻¹, 100 μmol·m⁻²·s⁻¹, and 400 μmol·m⁻²·s⁻¹. Three seedlings of both cultivars were used in each treatment for investigation. The shoot length, shoot number, and leaf number were measured on the 30, 60, 90, 120, and 150 days from August 15, 2014, as described in Experiment 2.

Next, the seedlings of both cultivars grown at the three different light intensities were continuously cultivated after the investigation period of 150 days. The number of days before flowering and the flowering rate were measured on June 21, 2015 (which was the 417 DAS for the ‘Misty’ seedlings), again as described in Experiment 2. In addition, the shoot length and leaf number per seedling-bearing bud were measured on January 27, 2015 (which was the 272 DAS for the ‘Misty’ seedlings) before the seedlings flowered. In this experiment, the number of seedlings investigated was as low as 3 due to the limited space in the controlled room.

Results

1. The effect of medium and light quality on the germination rate of blueberry seeds

The seed germination rate on the 35 DAS of blueberry seeds was assigned to an L8 orthogonal table (Table 1). While small differences were observed between cultivars, the medium and the light quality were found to have a major effect on germination rate. Moreover, no interaction between these factors was observed. Next, the transition of seed germination rate was analyzed (Fig. 1). The seed germination rate after 14 days from sowing in both cultivars was higher with the red LED irradiation treatment than with the blue LED irradiation treatment. In addition, when the seed germination rate in both cultivars of the WPM medium and the agarose medium was compared, both cultivars had higher germination rates on the WPM medium than on the agarose medium from 21 days to 35 days after sowing in the red LED irradiation treatment and on the 35 DAS in the blue LED irradiation treatment. In both cultivars, the highest seed germination rate was achieved when the WPM medium and the red LED irradiation were used.

2. The effect of different growth environments on growth and flowering of blueberry seedlings

The effect of different growth environments on the growth of blueberry seedlings is shown in Figure 2. By the 30 days after treatment, no significant differences were observed in the shoot length (Fig. 2A, D), shoot number (Fig. 2B, E), or leaf number (Fig. 2C, F) for the

Table 1. Effect of medium and light quality on the seed germination rate of blueberries ‘Misty’ and ‘Sharpblue’, analyzed on the 35 day after sowing by using the L8 orthogonal table.

<table>
<thead>
<tr>
<th>Factor*</th>
<th>Degree of freedom</th>
<th>Sum of squares</th>
<th>Unbiased variance</th>
<th>F value</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar (C)</td>
<td>1</td>
<td>16.66</td>
<td>16.66</td>
<td>0.28</td>
<td>ns</td>
</tr>
<tr>
<td>Light quality (L)</td>
<td>1</td>
<td>26934.00</td>
<td>26934.00</td>
<td>453.94</td>
<td>**</td>
</tr>
<tr>
<td>Medium (M)</td>
<td>1</td>
<td>726.00</td>
<td>726.00</td>
<td>12.24</td>
<td>**</td>
</tr>
<tr>
<td>C×L</td>
<td>1</td>
<td>16.66</td>
<td>16.60</td>
<td>0.28</td>
<td>ns</td>
</tr>
<tr>
<td>C×M</td>
<td>1</td>
<td>16.66</td>
<td>16.66</td>
<td>0.28</td>
<td>ns</td>
</tr>
<tr>
<td>L×M</td>
<td>1</td>
<td>6.00</td>
<td>6.00</td>
<td>0.10</td>
<td>ns</td>
</tr>
<tr>
<td>C×L×M</td>
<td>1</td>
<td>0.67</td>
<td>0.67</td>
<td>0.01</td>
<td>ns</td>
</tr>
<tr>
<td>Error</td>
<td>949.3</td>
<td>16.00</td>
<td>59.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Cultivar (‘Misty’ and ‘Sharpblue’), Light quality (red LED and blue LED), Medium (Agarose medium = Agarose and WPM medium = WPM).

ns and ** indicate not significant and significant at P<0.01 by ANOVA (n=3).
Fig. 1. Changes over time in the seed germination rates of ‘Misty’ (A) and ‘Sharpblue’ (B) blueberries cultured on agarose medium (Agarose) or WPM medium (WPM) under irradiation from red LED (λ = 630 nm) or blue LED (λ = 460 nm). Different letters and ns indicate significant differences and a non-significant difference at P < 0.05 by Tukey-Kramer test. Vertical bars indicate SE (n = 3).

Fig. 2. Effect of different growth environments on the growth of blueberry ‘Misty’ (A, B, C) and ‘Sharpblue’ seedlings (D, F, F). Seedlings were grown in the field condition as a control (○), in a controlled room (at a temperature of 9 to 22°C, relative humidity of 40 to 80%, and light intensity of about 300 μmol m⁻² s⁻¹) with the long-day condition (12-h photoperiod) (●), in a controlled room (at a temperature of 9 to 22°C, relative humidity of 40 to 80%, and light intensity of about 300 μmol m⁻² s⁻¹) with the short-day condition (8-h photoperiod) (●), and in the glasshouse (△). The growth of the seedlings was measured by the shoot length (A, D), the shoot numbers (B, E), and the leaf numbers (C, F). Different letters and ns indicate significant differences and a non-significant difference at P < 0.05 by Tukey-Kramer test. Vertical bars indicate SE (n = 5).
seedlings of both cultivars. However, significant differences were observed between the treatments on the 60 days after treatment. On the 150 days after treatment, seedlings incubated in the controlled room with a long day had grown to a significantly greater height than seedlings in the other treatments. The second largest plants were found in the controlled room with a short day and in the glasshouse. When these two treatments were compared, the shoot length (Fig. 2A) and the leaf number (Fig. 2C) of ‘Misty’ seedlings tended to increase slightly in the controlled room with a short day than seedlings in the glasshouse, although there was no significant difference. In the field condition, growth was slow and leaves were shed at low temperature by the 90 days after treatment, suggesting that the growth was the lowest of the four treatments.

In addition, when the seedlings of both cultivars in the controlled room with a short day were continuously cultivated, the number of days from sowing to flowering was 379 days for the seedlings of ‘Misty’, and 373 days for the seedlings of ‘Sharpblue’ (Table 2). On the other hand, the seedlings of ‘Misty’ that were moved from the controlled room with a long day to the controlled room with a short day flowered in 327 days, and the seedlings of ‘Sharpblue’ flowered in 357 days, indicating that the seedlings of both cultivars flowered early in the treatment that involved transfer of seedlings from the controlled room with a long day to the controlled room with a short day. The flowering rates of the seedlings were 100% for ‘Misty’ at the 421 DAS and for ‘Sharpblue’ at the 410 DAS, except for the 67% flowering rate shown by the ‘Misty’ seedlings that were moved from the controlled room with a long day to the controlled room with a short day. The seedlings grown in the controlled room with a short day and the seedlings that were moved from the controlled room with a long day to the controlled room with a short day started flowering from the tip of the shoot (Fig. 3B, C, G, H). Subsequently, flowering progressed to the basal part, and all of the flowers eventually formed normal fruits through artificial pollination (Fig. 3F, I). However, the seedlings of ‘Misty’ and ‘Sharpblue’ grown in the field condition (Fig. 3A), in the glasshouse (Fig. 3D) and in the controlled room with a long day (Fig. 3E), did not flower by the 421 DAS and the 410 DAS, respectively. In addition, when we investigated seedling growth for both cultivars just before flowering, the shoot length and the leaf number were significantly larger for the seedlings that were moved from the controlled room with a long day to the controlled room with a short day than the seedlings in the controlled room with a short day (Table 2).

### Table 2. Effect of different growth environments on the growth and flowering of blueberry ‘Misty’ and ‘Sharpblue’ seedlings.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Plant</th>
<th>Treatment</th>
<th>The number of days from sowing to flowering</th>
<th>Flowering (%)</th>
<th>Total shoot length (cm/plant)</th>
<th>Leaf number (number/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Misty</td>
<td>5</td>
<td>Field condition</td>
<td>—</td>
<td>0</td>
<td>24.0±4.0</td>
<td>40.0±6.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Controlled room with a long day (12 h)</td>
<td>—</td>
<td>0</td>
<td>250.5±7.5</td>
<td>140.5±37.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Controlled room with a long day (12 h) followed by a short day (8 h)</td>
<td>327.3±15.9</td>
<td>67</td>
<td>228.9±10.0</td>
<td>260.0±49.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Controlled room with a short day (8 h)</td>
<td>379.0±16.7</td>
<td>100</td>
<td>137.3±13.1</td>
<td>125.8±13.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Greenhouse</td>
<td>—</td>
<td>0</td>
<td>105.1±27.8</td>
<td>113.4±28.4</td>
</tr>
<tr>
<td>Sharpblue</td>
<td>5</td>
<td>Field condition</td>
<td>—</td>
<td>0</td>
<td>31.8±4.0</td>
<td>45.2±3.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Controlled room with a long day (12 h)</td>
<td>—</td>
<td>0</td>
<td>221.0±60.5</td>
<td>184.5±58.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Controlled room with a long day (12 h) followed by a short day (8 h)</td>
<td>357.6±10.8</td>
<td>100</td>
<td>188.0±27.9</td>
<td>192.0±35.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Controlled room with a short day (8 h)</td>
<td>373.8±5.5</td>
<td>100</td>
<td>89.7±15.3</td>
<td>84.4±15.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Greenhouse</td>
<td>—</td>
<td>0</td>
<td>115.7±24.4</td>
<td>138.4±28.0</td>
</tr>
</tbody>
</table>

* The day on which seedlings produced the first flower was the flowering day.

* The average number of days per plant, which was calculated as time taken for plants to flower from sowing ± SE ($n = 2, 3,$ or $5$).

* The flowering rate was investigated on August 18, 2016 (on the 421 day after sowing for the ‘Misty’ seedlings, and on the 410 day after sowing for the ‘Sharpblue’ seedlings) and calculated as (number of flowering plants/total number of plants) × 100.

* The shoot length and the leaf number were measured on May 7, 2016 before flowering (on the 321 day after sowing for the ‘Misty’ seedlings and on the 310 day after sowing for the ‘Sharpblue’ seedlings).
Next, flowering was observed for the ‘Misty’ seedlings grown at 300 μmol·m$^{-2}$·s$^{-1}$ and 400 μmol·m$^{-2}$·s$^{-1}$, while the ‘Misty’ seedlings did not flower at 100 μmol·m$^{-2}$·s$^{-1}$ (Table 3). The numbers of days from sowing to flowering in the ‘Misty’ seedlings grown at 300 μmol·m$^{-2}$·s$^{-1}$ and 400 μmol·m$^{-2}$·s$^{-1}$ were 329 and 300 days, respectively. Therefore, the seedlings seemed to flower earlier in the high light intensity conditions than the seedlings grown at low light intensity, although the difference was not shown to be statistically significant. We investigated the flowering rate of ‘Misty’ on the 417 DAS. The flowering rate of the ‘Misty’ seedlings cultivated at 300 μmol·m$^{-2}$·s$^{-1}$ was 67%, while the flowering rates of the ‘Misty’ seedlings cultivated at 400 μmol·m$^{-2}$·s$^{-1}$ were 100%. Furthermore, when the growth of seedlings just before flowering was examined, the shoot length and leaf number of the seedlings cultivated at 400 μmol·m$^{-2}$·s$^{-1}$ were greater than the seedlings cultivated at 300 μmol·m$^{-2}$·s$^{-1}$.

**Discussion**

In this experiment, medium and light quality had a major effect on the germination of blueberry seeds. No significant additive effect was observed when these factors were applied in combinations. As described in the experiments reported here, the germination rate was shown to be higher on the WPM medium than on the agarose medium. There are many other media that could be tested for *Vaccinium* germination. For example, Castro et al. (2012) reported that the seed germination of *V. meridionale* was suppressed with the concentration of salts in Murashige and Skoog medium (MS medium). Seeds of *V. myrtillus* germinated to a maximum of 88.9% on a modified MS medium with 9.1 μM Zeatin (Nin et al., 2017). On the other hand, when *V. corymbosum* seeds were sown on the WPM medium, the seed germination rate was 86.7% (Miyashita et al., 2009). Thus, a medium supplemented with inorganic salts and sucrose promoted seed germination in blueberries. However, it is necessary to determine the concentration range in which absorption of nutrients from the medium is not prevented. Traditionally, before blueberry seeds were sown, it was customary to keep the seeds in a refrigerator after extracting them from fresh blueberries (Shigyou et al., 2014). As observed in this experiment, sowing immediately after seed extraction from fruits can shorten the time required for obtaining the next generation (from getting seeds to flowering). In the experiments reported here, the seeds were sown in a WPM medium immediately after being extracted from the fruits, and were irradiated with a red LED. The seed germination rate was over 80% on the 35 DAS in both cultivars. This germination rate was higher than the germination rate reported by Miyashita et al. (2009), in which seeds were exposed to fluorescent light on a WPM medium. However, the light quality had more effect on germination than the type of medium. The result described here suggested that red light promoted the germination of blueberry seeds more...
effectively than blue light. Since similar results were obtained in previous reports (Giba et al., 1995), the light-induced seed germination by red light was here confirmed with the southern highbush blueberry. On the other hand, the germination rate on the 35 DAS under blue light in this experiment was as low as 10 to 20%. The promotion effect of blue light is expected to be small compared with the effect of red light. As the germination rate differed depending on the wavelength, the details must be investigated in the future. The effect of light and medium described here was the same for both cultivars tested, and no cultivar-specific effect was observed.

Next, the growth of seedlings was compared in different cultivation environments (Experiment 2). As a result, the growth achieved in the controlled room with a long day was significantly greater than that in the other three conditions examined. Among these three conditions, the seedlings maintained in the controlled room with a short day and in the glasshouse showed higher levels of growth than the seedlings in the field condition. The light intensity of the controlled rooms in both treatments was lower than that of the glasshouse, but the seedlings in both treatments kept growing without shedding leaves. In the field condition, the leaves were shed at low temperature and the seedlings were the smallest of the seedlings in all the treatments examined. This result suggests that, although the southern highbush blueberry used in this experiment sheds leaves at a low temperature in winter and stops growing, it has a characteristic of maintaining its growth when it is kept in a temperature range where it does not shed leaves (Horiuchi et al., 2013a, b). When the seedling growth was compared under different light intensities in the controlled room with a short day (Experiment 3), the shoot length, shoot number, and leaf number were the highest at 400 μmol·m⁻²·s⁻¹ (Fig. 4). Photoperiod and light intensity have been reported to affect the seedling growth in previous studies. In the lettuce, garland chrysanthemum (Chrysanthemum coronarium L.) and tomato (Solanum lycopersicum L.), growth increased with increasing light intensity of the supplemental lighting (Fukuda et al., 2000). When the photoperiod was extended compared to the natural condition, it significantly increased the elongation of the primary shoot and the number of leaves on lateral shoots per main shoot in potted grapes (Kubota et al., 2001). The growth of secondary shoots increased as the light intensity increased in Japanese persimmon trees under a long day photoperiod condition (Kurahashi et al., 2002). Vegetative growth of the southern highbush blueberry was greater in a 16-h photoperiod than in an 8-h photoperiod (Spann et al., 2003). These results suggest that photoperiod and light intensity are the most influential physical factors in the growth of blueberry seedlings. However, further research using a larger set of plants is required in order to fully validate these results (Experiments 2 and 3).

There are two methods for shortening the juvenile phase. One is to transfer seedlings to an environmental condition where flower bud initiation and flowering are induced, after a sufficient level of seedling growth is obtained by promoting its growth. The other is to cultivate the seedlings in the environmental conditions that
Table 3. Effect of the difference in light intensity on the growth of blueberry ‘Misty’ seedlings.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Treatment</th>
<th>The number of days from sowing to flowering</th>
<th>Flowering (%)</th>
<th>Total shoot length (cm/tree)</th>
<th>Leaf number (number/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 μmol·m⁻²·s⁻¹</td>
<td>—</td>
<td>0</td>
<td>64.8 ± 18.4</td>
<td>60.0 ± 24.4</td>
</tr>
<tr>
<td>2</td>
<td>300 μmol·m⁻²·s⁻¹</td>
<td>329.0 ± 2.0°</td>
<td>67</td>
<td>79.6 ± 12.1</td>
<td>76.0 ± 7.0</td>
</tr>
<tr>
<td>1</td>
<td>400 μmol·m⁻²·s⁻¹</td>
<td>300.0 ± 6.4</td>
<td>100</td>
<td>219.3 ± 29.3</td>
<td>116.3 ± 18.4</td>
</tr>
</tbody>
</table>

The day on which seedlings produced the first flower was the flowering day.

The flowering rate was investigated on June 21, 2015 (on the 417 day after sowing for the ‘Misty’ seedlings) and calculated as (number of flowering plants/total number of plants) × 100.

The flowering rate was calculated as time taken for plants to flower from sowing ± SE (n = 3).
trolled room for 10 months for maximum growth and then moved to a short-day controlled room. Furthermore, when cultivated under the short day condition at 400 μmol·m⁻²·s⁻¹ for the entire length of the experiment, flowering of ‘Misty’ seedlings was observed within 300 DAS. Thus, we conclude that the use of a plant factory can lead to acquisition of the next generation of blueberry fruits significantly earlier, and that it is possible to breed blueberries in as short as a one year cycle.

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