Effect of Blue-Light PPFD Percentage in Red and Blue LED Low-Light Irradiation during Storage on the Contents of Chlorophyll and Rubisco in Grafted Tomato Plug Seedlings

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Grafted tomato (Lycopersicon esculentum Mill.) plug seedlings were stored at 10°C for 21 days under a photosynthetic photon flux density (PPFD) of 2 µmol m⁻² s⁻¹ using mixed light from red and blue LEDs, with different percentages of blue-light PPFD (0, 2, 5, 10 and 50%). The effect of blue-light PPFD percentage was investigated and the optimal percentage was determined based on the contents of chlorophyll (Chl) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in leaves on the last days of storage. Chl and Rubisco contents decreased irrespective of the blue-light treatment during storage. On the last day of storage, Chl content were greater in the 2, 5, and 50% blue-light treatments than in the 0% treatment and Rubisco content were greater in the 2-50% treatments than in the 0% treatment. The suppression of the decrease in the Chl content contributed to the preservation of the visual quality of the stored seedlings. It can be concluded that the 2, 5 and 50% blue-light PPFD were effective in preserving the quality of grafted tomato seedlings during storage at 10°C and 2 µmol m⁻² s⁻¹. In a commercial operation, 2% blue-light PPFD would be optimal, because blue LEDs are expensive.

Keywords : blue light, light emitting diode, low temperature storage, red light, tomato seedlings

INTRODUCTION

Low-light irradiation at photosynthetic photon flux densities (PPFD) of 1-30 µmol m⁻² s⁻¹ during low temperature storage prolongs storability of Pelargonium cuttings (Paton and Schwabe, 1987). Heins et al. (1994) also reported that low-light irradiation could preserve plug seedling quality during low temperature storage of tomato and 18 other species of bedding plants. Further studies have suggested that a light intensity at which the net photosynthetic rate was maintained at zero (the light compensation point) was needed to suppress changes in dry weight and to preserve quality of in vitro broccoli plantlets (Kubota and Kozai, 1994) and eggplant plug seedlings (Kozai et al., 1996) during low temperature storage. In these studies, white fluorescent lamps were used as a light source. Fujiwara et al. (1999, 2001a, b) suggested that red light-emitting diodes (LEDs) were more appropriate than white fluorescent lamps as a light source for low-light irradiation during low temperature storage, because the lifetime of LEDs is longer than those of white fluorescent lamps, and PPFD can easily be controlled at the plant canopy level by regulating the supplied voltage. Fujiwara et al. (2003) reported that irradiation using a mixture of red and blue light (RB-light)
from LEDs improved the quality of tomato plug seedlings on the last day of storage in terms of leaf area and visual quality, such as color of leaves and stems and extent of necrosis compared with those by irradiated by red light alone (R-light). We observed suppression of the decrease in the amounts of photosynthesis-related components, such as ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and chlorophyll (Chl), of grafted tomato seedlings during low temperature storage following irradiation by RB-light from LEDs in comparison to irradiation by R-light (Kaneko-Ohashi et al., 2004). This means that irradiation by RB-light suppresses the decrease in photosynthetic capacity of leaves in the seedlings during low temperature storage.

The amount of protein in a leaf is the result of a balance between its synthesis and degradation. We speculate that the amounts of Chl and Rubisco synthesized were greater under irradiation by RB-light than under irradiation by R-light, which led to the suppression of the decrease in the amounts of Chl and Rubisco of tomato seedlings during low temperature storage. Sæbø et al. (1995) observed that the Chl content was greater in birch plantlets grown under blue light than under red light, and suggested that blue light was important for Chl synthesis and plastid development. Sawbridge et al. (1994) reported that the level of expression of rbcS, the gene encoding the small subunit of Rubisco, in bean leaves grown under blue-enriched light irradiation was much greater than that under red light irradiation. On the other hand, little is known about how light quality affects the degradation of Chl and Rubisco in leaves.

We conclude that blue light mixing was effective for maintaining visual quality in terms of color of leaves due to the greater Chl content during low temperature storage of grafted tomato seedlings. On the other hand, using only blue LEDs or a large number of blue LEDs together with a small number of LEDs of another color was not practical, because blue LEDs are still expensive. The price of blue LEDs is roughly 4-5 times higher than that of red LEDs. Therefore, the mixing ratio of blue LEDs should be a comparatively low ratio.

The aim of this study was to determine the optimal blue-light PPFD percentage for suppression of the decrease in the amounts of Chl and Rubisco and preservation of the visual quality of grafted tomato plug seedlings during low temperature storage under low irradiation.

**MATERIALS AND METHODS**

Plant materials: Grafted tomato (scion: *Lycopersicon esculentum* Mill. cv. House Momotaro; root stock: cv. Kagemusya) plug seedlings in a 72-plug tray were obtained from a commercial seedling supplier (Yamaguchi Engei Co., Ehime, Japan). Before storage, the seedlings were incubated at 27±1°C and under 230±30 μmol m⁻² s⁻¹ PPFD for 24 h. Out of 72 seedlings, 54 (nine seedlings X six groups) were selected and evenly split into six storage treatments. One set of nine seedlings (one group) was analyzed before storage (on day 0 after storage). The nine seedlings for each storage treatment were placed in a separate 9 (3 X 3)-plug tray of the same size as the 72-plug tray.

Storage conditions: Based on previous storage experiments by Fujiwara et al. (2003), seedlings were stored in a transparent acrylic resin case (500 mm wide X 345 mm deep X 300 mm high) at 10±0.5°C and >95% relative humidity under 2 μmol m⁻² s⁻¹ PPFD at the canopy level of the seedlings for 21 d. PPFD was set up with a PPFD meter (LI-190SA with LI-250; Li-Cor, Lincoln, Nebraska) and an illuminometer (T-1M; Minolta, Tokyo) according to the methods of Fujiwara et al. (1997). Six different storage treatments were prepared using R-light and RB-light from LEDs with different percentages of blue-light PPFD (0, 2, 3, 5, 10 and 50%) (Table 1). The relative spectral photon-number distributions of the red and blue LEDs are shown in Fig. 1. The blue light slightly contains a green light of 500–550 nm, the ratios of green-light PPFD to total PPFD (2 μmol m⁻² s⁻¹ PPFD) were 0.06%, 0.09%, 0.15%, 0.3% and 1.5% in the 2% to 50% treatments, respectively. During storage, the seedlings were irradiated for 24 h continuously. Before storage and
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Table 1 Blue-light PPFD ratio and storage conditions for each treatment.

<table>
<thead>
<tr>
<th>Blue-light PPFD ratio (%)</th>
<th>Red light PPFD (µmol m⁻² s⁻¹)</th>
<th>Blue light PPFD (µmol m⁻² s⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>2.00</td>
<td>0</td>
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<tr>
<td>2</td>
<td>1.96</td>
<td>0.04</td>
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<td>5</td>
<td>1.90</td>
<td>0.10</td>
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<tr>
<td>10</td>
<td>1.80</td>
<td>0.20</td>
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<tr>
<td>50</td>
<td>1.00</td>
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</table>

' Photosynthetic photon flux density at the top of the grafted tomato plug seedlings.

Fig. 1 Relative spectral photon-number distribution of the red light-emitting diodes (upper panel) and the blue light-emitting diodes (lower panel).

after 21 d of storage, all three leaves of the four seedlings from each storage treatment were collected. The leaves were weighed and the leaf area measured, and they were stored at -85°C until analysis.

Determination of Chl and Rubisco: The leaf was homogenized with a chilled mortar and pestle in 50 mM Na-phosphate buffer (pH 7.0) containing 2 mM Na-iodoacetate, 0.8% (v/v) 2-mercaptoethanol, 1 mM phenylmethane sulfonyl fluoride (PMSF), 1% (w/v) polyvinylpolypyrrolidone and 5% glycerol. Chl and Rubisco contents were measured according to the methods of Makino et al. (1994). A calibration curve for Rubisco determination was obtained with Rubisco purified from tomato leaves.

RESULTS AND DISCUSSION

Leaf fresh weight of tomato plug seedlings after 21 d storage tended to be smaller than that of those before storage, although there was no significant difference among in the 10 and 50% blue-light PPFD treatments and in the BS (Table 2). The ratio of leaf fresh weight of seedlings after storage to that of seedlings before storage was 72–88%. Based on the method of Fujiwara et al. (2001a), the light compensation point (the light intensity at which the net photosynthetic rate was maintained at zero) was set at 2 µmol m⁻² s⁻¹ during storage in this study. However, fresh weight losses during storage indicated that the light compensation point of seedlings in this experiment may be higher than 2 µmol m⁻² s⁻¹. Recently, Fujiwara et al. (2005) pointed out that the light compensation point potentially differs with growth stage and condition. In addition, although
relative humidity inside the acrylic resin case was more than 95% during storage, water content of the seedlings may decrease irrespective of the blue light PPFD ratio. After 21 d storage, there was no significant difference in leaf fresh weight among the blue-light PPFD treatments although the mean value of the 10 and 50% treatments was larger than those in the 0, 2 and 5% treatments. Blue light generally promotes stomatal opening more than other light wavelengths (Sharkey and Raschke, 1981) and the increase in the photosynthetic rate by increased stomatal conductance may be related to the relatively larger biomass at higher blue-light PPFD ratio. Leaf area of the stored seedlings was not significantly different among blue-light PPFD treatments, although leaf area shrank during storage regardless of treatment. All seedlings had three leaves before and after storage. Plant length also tended to be almost the same before storage and after 21 d storage (data not shown).

Figure 2 shows the Chl content per total leaf area of tomato plug seedlings before storage and after 21 d storage. The dotted line represents the mean value of Chl content before storage. Chl content in the leaves of tomato plug seedlings after 21 d of storage was smaller than that of those before storage irrespective of the blue light PPFD ratio. The ratio of Chl content in leaves of seedlings after storage to that of seedlings before storage was 69-82%, suggesting that Chl content was progressively decreasing during low temperature storage under low-light irradiation. After 21 d of storage, Chl content tended to be greater in the 2–50% blue-light PPFD treatments than in the 0% blue-light PPFD treatments, although there were no significant differences between the 0 and 10% treatments. Furthermore, the Chl content at each leaf position (1st to 3rd leaves) of stored seedlings also tended to be greater in the 2–50% blue-light PPFD treatments than in the 0% treatments (data not shown). These results suggested that irradiation of RB-light compared with R-light should suppress the decrease in Chl content. According to our previous report (Kaneko-Ohashi et al., 2004), Chl content tended to be greater with increase in the blue-light PPFD ratio (0, 3 and 50% blue-light PPFD ratio) after 21 d storage, although there was no significant difference among the blue-light treatments. However, in this study, such a relationship between the Chl content and the blue-light PPFD ratio was not observed. We concluded that the 2, 5 and 50% treatments were effective. In addition, Rubisco content in leaves of tomato plug seedlings after 21 d of storage were greater in the 2–50% blue-light PPFD treatments than in the 0% treatments (Fig. 3). No relationship between the Rubisco content and the blue-light PPFD ratio was observed. We speculate that the synthesis of photosynthesis-related key components, including Chl and Rubisco, was promoted by RB-light more than by R-light, which led to suppression of the decrease in the amounts of Chl and Rubisco. In previous reports, the difference in the contents of Rubisco and Chl in leaves caused by different blue-light PPFD ratios did not affect the initial growth during cultivation following storage (Kaneko-Ohashi et al., 2004). However, blue light mixing was effective for maintaining visual quality in terms of color of leaves due to the greater Chl content following low temperature storage.
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Fig. 2 Chlorophyll content in leaves of grafted tomato plug seedlings after 21 d of storage. The dotted line represents the mean value of Chlorophyll content in leaves before storage. Vertical bars represent standard errors of the means (n=4). Means with different letters were significantly different at the 5% level by HSD tests.

Fig. 3 Rubisco content in leaves of grafted tomato plug seedlings after 21 d of storage. Samples were the same as those in Fig. 2. Vertical bars represent standard errors of the means (n = 4). Means with different letters were significantly different at the 5% level by HSD tests.

of grafted tomato seedlings.

To summarize the results, the biomass and the morphology of the tomato plug seedlings after 21 d of storage seemed unaffected by the blue-light PPFD ratio (Table 2). The 2, 5 and 50% blue-light PPFD treatments were effective to suppress the decrease in Chl content (Fig. 2). Therefore, we conclude that 2, 5 and 50% blue-light PPFD were the most effective percentages of those tested for preserving the quality of grafted tomato seedlings during low-light irradiation storage at 10°C and 2 μmol m⁻² s⁻¹ PPFD. However, in a commercial operation, 2% of blue-light PPFD would be the optimal percentage because blue LEDs are expensive. We will next examine whether the blue light PPFD ratio can be lowered below 2%.

REFERENCES


